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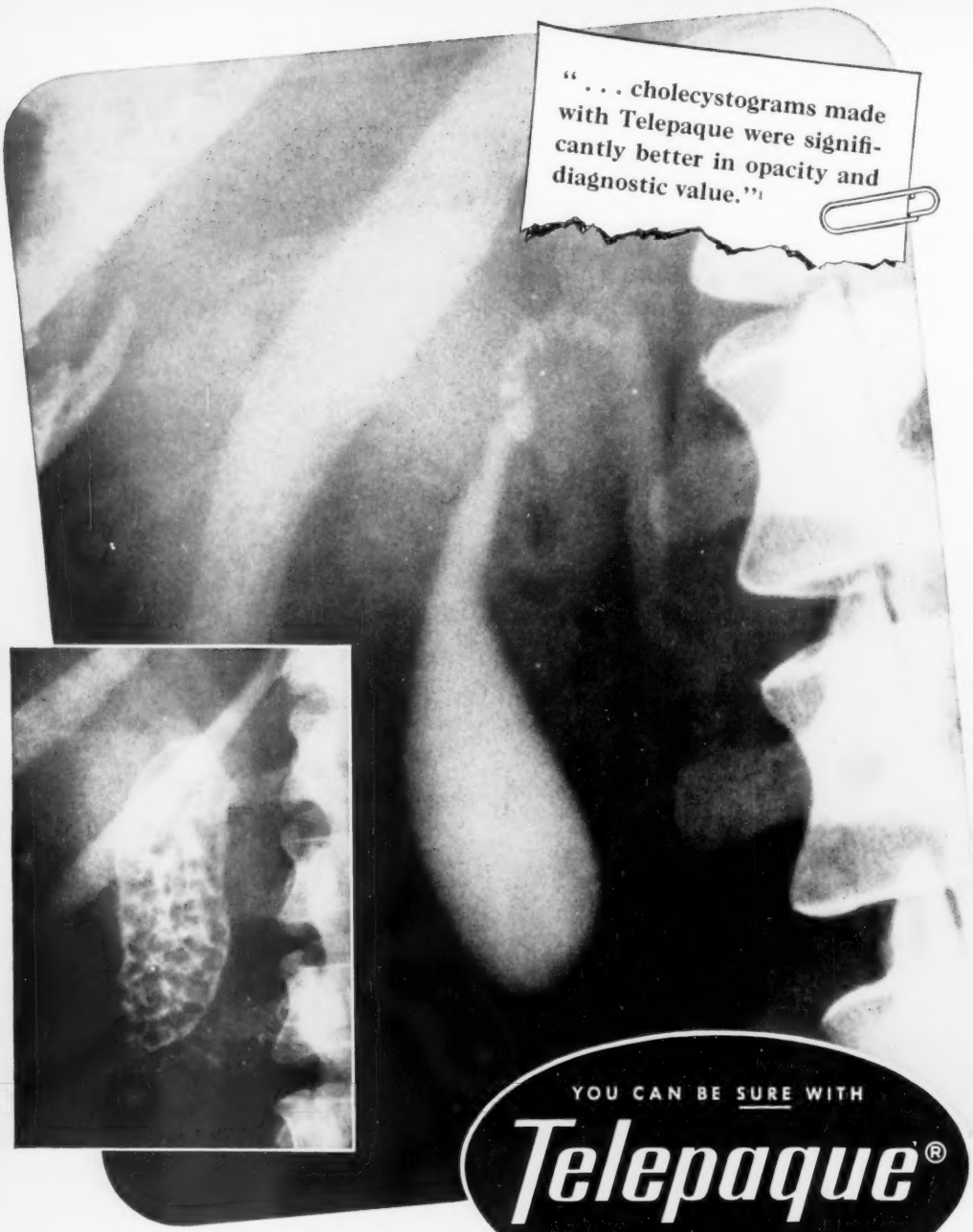

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CIRCULATORY BY-PASS OF THE RIGHT HEART. I. PRELIMINARY OBSERVATIONS ON THE DIRECT DELIVERY OF VENA CAVAL BLOOD INTO THE PULMONARY ARTERIAL CIRCULATION. AZYGOS VEIN—PULMONARY ARTERY SHUNT**

The surgical treatment of certain congenital anomalies of the right side of the heart characterized by mal- or non-function of the right auricle or right ventricle or both may depend on the possibility of the direct delivery of blood from the vena cava into the pulmonary arterial circulation.†

The immediate and ultimate success of this procedure would depend on the volume of blood flow through the pulmonary artery. As stated by Dexter, *et al.*,[1] "The volume of blood flowing through the pulmonary artery per unit of time is dependent upon the pressure in the pulmonary artery, the resistance to blood flow offered by the pulmonary vasculature, and the ability of the left side of the heart to handle the volume of blood which it receives." On the basis of studies by these investigators on the pulmonary artery pressure in normal adult humans, a mean pressure of about 13 to 17 millimeters of mercury would be sufficient to propel a normal blood flow through the pulmonary artery at rest. A venous pressure of this magnitude would, acting alone, usually be insufficient to produce peripheral edema.[2] Prolonged increased venous pressure may, however, lead to varicosities and the development of a collateral venous circulation.

METHODS

Mongrel dogs were anesthetized with nembutal and using sterile technique the azygos vein on the right side was divided and the peripheral end ligated. The central end was trimmed close to the superior vena cava, its connection with the cava being temporarily occluded by a curved Potts' bulldog type clamp. An anastomosis was then performed

* James Hudson Brown Memorial Fund Research Fellow in Surgery and Pathology.

** With the technical assistance of John E. Fenn. Aided in part by Grants from the Public Health Service (H-851 C4) and the Victoria Fund for Cardiovascular Research at Yale University.

† In a paper delivered before the Forum on Fundamental Surgical Problems, Warden, *et al.*,[4] described experiments where they anastomosed the right auricle to the main pulmonary artery for the purpose of by-pass of the right ventricle. H. B. Shumacker in discussion of this paper indicated that experiments in his laboratory were being carried out where the venae cavae were anastomosed directly to the pulmonary artery.

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between the stump of the central end of the azygos vein and the distal end of the divided right main pulmonary artery. The cardiac end of the right pulmonary artery was ligated. Following the completion of the anastomosis, the superior vena cava was occluded as it entered the right heart by a double ligature of heavy silk. In one animal the anastomosis was made between the distal end of the divided right pulmonary artery and the inferior vena cava.

Studies in the majority of these experiments included: venous pressure recorded from the superior vena cava, (i) before ligation of the cava, (ii) after ligation of the cava and before opening of the anastomosis to the pulmonary artery, and (iii) after opening of the anastomosis to the pulmonary artery. Oxygen studies (content, capacity, saturation) were done on the blood obtained from the superior vena cava and the aorta, (i) before ligation of the cava and (ii) after ligation of the cava with the

TABLE 1. VENOUS PRESSURE STUDIES

	Pressure superior vena cava mm. saline		
		After ligation SVC azygos-PA shunt	
Experiment No.	*Initial*	*Closed*	*Open*
1	60	400	250
2	61	225	180
3	30	245	180
6	<30	165	134
7	30	600	214
8	<25	300	118
9	<30	477	145

anastomosis of the cava to the pulmonary artery open. A venogram of the superior cava was performed in four experiments two to three weeks following operation, and in one of these it was performed a second time 73 days following operation; 15 to 25 cc. of a 70% solution of Diodrast were injected into either a vein in the foreleg or the external jugular vein in approximately five seconds.

RESULTS

The results of this investigation are based on nine experiments where studies were made.

Venous pressure. (Table 1). The pressure in the superior vena cava was low (30-60 millimeters of saline) prior to ligation of the superior vena cava but rose rapidly on complete caval occlusion at the level of the right heart (165-600 millimeters of saline). Following opening of the anastomosis between the superior cava and the right pulmonary artery the pressure in the occluded superior cava invariably fell as blood passed into the pulmonary arterial circuit of the right lung. After opening of the anastomosis

FIG. 1. Angiocardiogram made in four experiments. Experiment 1 (upper left) 73 days postoperatively. Experiment 2 (upper right) 43 days postoperatively. Experiment 7 (lower left) 21 days postoperatively. Experiment 8 (lower right) 16 days postoperatively.

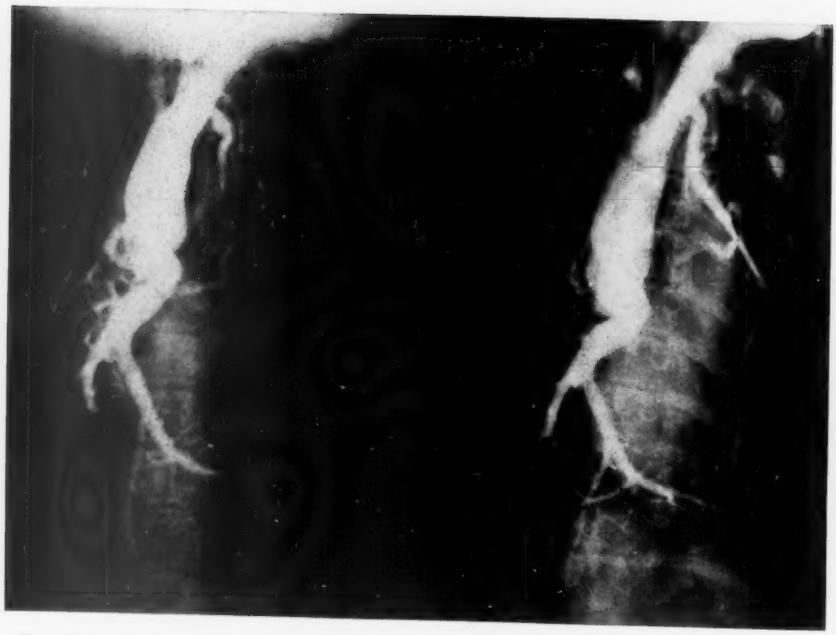

FIG. 2. Angiocardiogram made in Experiment 1, 18 days postoperatively (left) and 73 days postoperatively (right).

the pressure in the superior cava varied from a minimum of 118 millimeters of saline (Experiment 8) to a maximum of 250 millimeters of saline (Experiment 1). The percentage of fall varied from 18.8 (Experiment 6) to 69.6 (Experiment 9). In a few experiments vena caval pressures were repeated at a later time. In Experiment 1 the pressure in the external jugular vein was found to be 68 millimeters of saline and 65 millimeters of saline on the 73d and 88th days following operation as compared with a superior caval pressure of 250 millimeters of saline on the day of operation. The external jugular pressure in Experiment 2 was 140 millimeters of saline 43 days after operation as compared with 180 millimeters of saline in the superior cava on the day of operation. In Experiment 7 the pressure in

TABLE 2. OXYGEN STUDIES

	Blood oxygen per cent saturation azygos-PA shunt			
*Experiment No.**	*Pre-*	*Post-*	*Pre-*	*Post-*
	Aorta		*Superior vena cava*	
6	96.5	99.4	48.5	19.2
7	98.7	99.8	92.1	89.9
8	97.4	100+	88.8	70.2
9	100+	100+	68.7	64.5

* All animals breathing 100% oxygen.

the superior vena cava 21 days after operation was almost the same as on the day of operation.

Oxygen studies. (Table 2). These studies usually revealed a fall in the oxygen saturation of the venous blood in the superior vena cava after the anastomosis of the cava to the pulmonary artery had been opened for 5 to 20 minutes. There was no significant change in the saturation of the arterial blood (aorta).

Angiocardiography. (Fig. 1 and 2). In four experiments angiocardiography was carried out in the postoperative period. In all experiments the pulmonary vascular tree on the right side was clearly visualized. In one animal a delay in emptying of the dye from the right upper lobe was noted.

COMMENT

The results of these early experiments suggest that an anastomosis between the superior vena cava and the distal end of the right pulmonary

artery can be expected to remain patent in the majority of animals. The evidence suggests that at least some blood from the superior vena cava passes through the right lung. The evidence also suggests that there may be a difference in pulmonary arterial resistance from animal to animal under the imposed experimental conditions. There is some suggestion (Experiment 1) that there may actually be a lowering of pulmonary arterial resistance after the relatively prolonged exclusion of the pulmonary artery from direct continuity with the right heart. Whether this is an expression of decreased pulmonary vascular resistance or decreased flow through the shunt due to an expanding collateral venous circulation between the superior and inferior venae cavae remains to be determined. The serial angiocardiograms taken on this animal at intervals of 55 days (Fig. 2) do not suggest, however, the formation of extensive collateral venous circulation.

By-pass of the right heart may be accomplished by the anastomosis of the inferior and superior venae cavae to the right and left pulmonary arteries. By-pass of the right ventricle alone can be accomplished by the anastomosis of the right auricle to the main pulmonary artery.[3] Our interest in attempts to by-pass the entire right heart developed following unsatisfactory results in several patients with tricuspid stenosis on whom a systemic artery-to-pulmonary-artery shunt had been performed. In these patients there usually exists an interauricular septal defect as well.

The cardiac anomalies which might benefit from this operation include stenosis or atresia of the tricuspid and pulmonary outflow tracts. By-pass of the right side of the heart may also be indicated where a poorly functioning right ventricle, although there is no obstruction to the outflow, accompanies an anomaly in the position of the tricuspid valve (Epstein's anomaly). Further, when there is a single ventricle or a bilocular heart, the direct passage of blood from the cavae to the pulmonary arteries may be beneficial. Possibly, by-pass of the right side of the heart may be indicated in conditions characterized by pulmonary hypertension, particularly Eisenmenger's syndrome, where the hypertension has not become irreversible. Also, in transposition of the great vessels, by-pass of the right heart may be beneficial, especially if there is an associated pulmonary stenosis. By-pass of the right heart may also be indicated where there is obstruction of the cavae where they join the heart, or where there is an abnormal insertion of either cavae into the left auricle.

Further experiments are in progress with two main purposes: (i) to determine if the entire venous circulation can be made to by-pass the heart directly into the lungs, and (ii) to investigate changes in pulmonary vascu-

lar resistance with part or all of the venous blood made to by-pass the right heart.

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SOME REMARKS ON DIABETIC ACIDOSIS*

Whether or not, as Pope said, "the proper study of mankind is man," the egocentricity and introspective preoccupation of *genus homo* has made this study his favorite indoor sport. Although he is as filled with general insatiable curiosity as Kipling's elephant child, this self-preoccupation has tended to orient this curiosity in the direction of his own experiences and especially towards the escape from labor and suffering and the acquisition of power and possessions. It is not strange, therefore, that the phenomena of disease have been so often the initial stimuli to fundamental biological investigations and discoveries. This has been particularly true in the fields of physiology and biochemistry. These sciences owe much to astute observations and inferences of members of the medical profession. It is hard to imagine what our knowledge of intermediary metabolism would be today if its investigation had not been so largely dominated by its origin in the mysteries of diabetes. The discovery by v.Mehring and Minkowski that a similar condition could be produced in other animals, especially the docile dog, by removal of the pancreas, permitted the physiologists to exploit the disease. Although this contributed greatly to fundamental knowledge of both intermediary metabolism and diabetes, the literal translation of phenomena observed in an omnivorous animal into terms of the reactions of a carnivorous animal inured to a diet lacking preformed carbohydrate led to a variety of erroneous conclusions. Particularly it tended to center interest upon the combustion of carbohydrate and to divert it from the other disorders that are found in human diabetes, especially the disorder of fat metabolism manifested in ketosis and its consequences.

In a highly complex organism it is seldom possible to disturb one set of equilibria without affecting others. It was early recognized that the phenomena of human diabetes, especially of ketosis, extended beyond the boundaries of intermediary metabolism into the provinces of acid-base and electrolyte equilibria and water exchange. Since these secondary disturbances do not, however, attain the magnitude in convenient experimental animals that they do in man, their elucidation has been largely the task of

* Delivered at Medical Conference, Yale University School of Medicine, October 28, 1954.

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clinicians. In the continuing cycle of exchange these contributions of medicine have opened new vistas for physiological exploration.

Although I was at that time ostensibly studying cardiac dyspnea, the first paper that I published on my own, in 1917, dealt with the disorders of acid-base equilibrium and respiratory exchange in diabetic acidosis. Although my major scientific interest has never been diabetes per se, I find that since that time I have been guilty of publishing 22 articles on the subject, 15 of which dealt particularly with acidosis. The information to be culled from this source seems inexhaustible and its clinical implications of prime significance. I suspect that there will be further lessons to be learned from it by my grandchildren.

Diabetic acidosis is the natural consequence of the inability of an animal that is conditioned to subsist on a carbohydrate or mixed diet to oxidize carbohydrate; ketosis may be regarded as an adaptation to deficient carbohydrate combustion. Although it has been known for a long time that fat can be formed from carbohydrate, it has only recently been recognized that this conversion is not an occasional process, usually confined to fattening pigs and gluttons, but a normal route for the utilization of carbohydrate; that fat is probably the chief source of the energy used in ordinary life. Conversion of carbohydrate to fat, however, requires the expenditure of a certain amount of energy and cannot, therefore, be effected if there is no carbohydrate available or if carbohydrate cannot be oxidized.

Figure 1 is a rough diagrammatic attempt to outline some of the major steps of intermediary metabolism.[7] The circle at the bottom represents the Krebs' cycle, the device or burner in which products derived from the foodstuffs are oxidized to provide energy for vital activities. The cycle which is depicted as moving clockwise begins and ends with oxaloacetic acid. This means that any compound that enters must be completely consumed in the cycle. Oxaloacetate may react with pyruvic acid, a 3-carbon group, to form the next member of the cycle, a 6-carbon group, after the initial loss of one carbon. A properly constituted 2-carbon group can serve the purpose equally well. In either case in its course around the cycle the added compound is completely oxidized to CO_2 and H_2O. Both fatty acids and carbohydrate in preparation for combustion form 2-carbon groups; but apparently, in the absence of carbohydrate products or the ability to burn carbohydrate products because insulin is lacking, the device breaks down or is clogged. To maintain the cycle, only a small amount of carbohydrate need be burned and this is not dependent upon the total amount of fuel oxidized. This modicum of carbohydrate is not, therefore, required for energy production, but to keep the burner going. If it is not provided, the formation of fat from carbohydrate is impaired. It was quite a shock to learn that one of

the chief actions of insulin is the potentiation of this conversion. In addition, 2-carbon fractions formed from fatty acids are coupled in the liver to form the 4-carbon ketone bodies, acetoacetic and β-hydroxybutyric acid which are burned in the tissues. In diabetic acidosis, since utilization of carbohydrate is reduced to a minimum, the whole burden of energy production

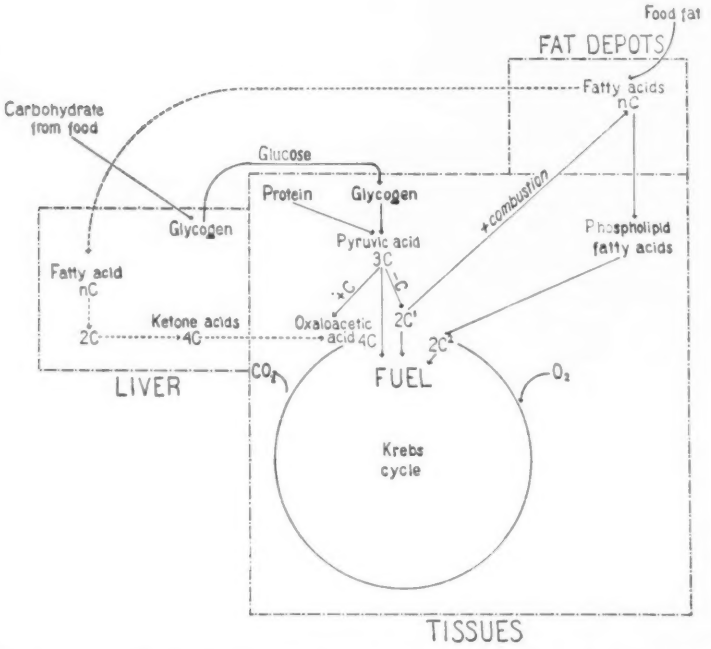

FIG. 1. A schematic representation of the interrelationships of the foodstuffs in intermediary metabolism.

falls upon fat. The combustion of ketones, however, accounts for only a fraction of the total fat consumed. The remaining energy is, therefore, presumably derived from combustion of the 2-carbon derivatives of fatty acids in the tissues. That these are indeed consumed in the Krebs' cycle has been demonstrated. It has also been shown that the ketone bodies are oxidized in this cycle. It has, therefore, been proposed, but not conclusively proved, that the 4-carbon ketone bodies are used to replace the 4-carbon oxaloacetic acid when products of carbohydrate metabolism are not available, thereby maintaining the integrity of the Krebs' burner.

Under these circumstances the demand for carbohydrate is greatly increased while its disposal is diminished in two ways: it can neither be burned nor converted to fat. Not only is glycogen broken down, but the destruction of protein increases, adding further to the load of glucose. Blood sugar rises to phenomenal heights and glycosuria ensues with consequent diuresis. The production of ketone bodies also rises, exceeding the capacity of the tissues to oxidize them. Either the liver is overgenerous in its anxiety or ketone bodies are less efficiently used than the usual products of carbohydrate metabolism that they are replacing. Since these ketone bodies are relatively strong acids they displace bicarbonate from combustion with the sodium of the extracellular fluids, producing acidosis and dyspnea.

The transfer from a food mixture containing carbohydrate to one that consists entirely of protein and fat entails a major adaptive change in the procession of chemical reactions that constitute intermediary metabolism. Reversal of this revolutionary change requires a certain amount of time. If a person is subjected to starvation or carbohydrate starvation, the respiratory quotient after an interval approaches 0.70, indicating that energy is being derived chiefly or entirely from fat. At the same time ketosis appears. If, under these circumstances, a dose of glucose is taken, the respiratory quotient does not rise for an appreciable interval; the blood sugar rises excessively and remains elevated unduly long; sugar may appear in the urine. If insulin is given, its action is delayed and its effects on the blood sugar are smaller. The effects of carbohydrate starvation are exaggerated or accelerated by exercise because, besides accelerating the combustion of carbohydrate, insulin promotes formation of fat from carbohydrate and retards the formation of carbohydrate from protein. The starvation diabetes produced by insulin does not, of course, begin when insulin is given; at that time the oxidation of carbohydrate is accelerated. It follows hypoglycemia, which marks the exhaustion of endogenous stores of carbohydrate. In contrast, the utilization of carbohydrate is greatly augmented by the administration of carbohydrate. All these phenomena are exaggerated in the diabetic subject, in whom the combustion of carbohydrate is already impaired.[6]

If diabetic ketosis and acidosis are natural consequences of the inability to oxidize carbohydrate, they cannot be attributed to overindulgence in carbohydrate food per se. On the other hand, ketosis and acidosis should follow protracted periods of hypoglycemia from insulin. As a matter of fact, as the result of prolonged investigation of starvation phenomena in the diabetic, we have come to the conclusion that a large proportion of acidotic episodes, especially in children and unstable adults, are precipitated by hypoglycemic incidents. Vomiting, since it prohibits provision of carbo-

hydrate, is another chief offender. It may be a precipitating cause of diabetic acidosis or an aggravating element in its development. The other main causes of acidosis are infections and omission of insulin, which primarily impair the combustion of sugar. With these considerations of the nature and causes of ketosis in mind, it should be possible to forestall a large proportion of acidotic episodes.

A certain proportion of the disorders that make up the picture of acidosis precede the development of this condition, arising from the hyperglycemia and profuse glycosuria that usher it in. Among these are wastage of salt and the reductions of sodium and chloride in the serum that have long been recognized as characteristics of fully developed acidosis. The mechanism by which they are produced was defined by Seldin and Tarail.[8] Since the cells of the body are not freely permeable to glucose, the effective osmotic pressure of the extracellular fluid increases when the blood sugar rises. (The term *effective osmotic pressure* is applied to the osmotic pressure of those components that do not traverse cellular membranes and therefore determine the distribution and transfers of water between the cells and the extracellular fluid.) Ordinarily the effective osmotic pressure is produced almost entirely by sodium salts; but glucose, other saccharides, and mannitol contribute to it when they accumulate in the extracellular fluid. The increase of effective osmotic pressure owing to the accumulation of such substances draws water from the cells, which consequently become dehydrated. The water thus withdrawn from the cells dilutes the extracellular fluid, causing the concentrations of sodium and chloride in the serum to fall. Part of the reduction of sodium and chloride in the serum in diabetic acidosis must be attributed not to the loss of these ions in the urine, but simply to dilution by this water derived from the cells. This reaction is illustrated in Figure 2, constructed from data of Seldin and Tarail.[8] The total height of each column represents the osmolar concentrations of sodium salts and glucose combined, the upper black segment representing the contribution of glucose. In each triad of columns the left-hand column is the initial or normal pattern of the serum in which glucose plays a negligible part. The second column shows the effect of raising the concentration of glucose by intravenous injection of 25 per cent glucose solution.

In the first experiment the concentration of glucose in the serum was raised to about 800 mg. per cent. The total osmolar concentration rose sharply, but the concentration of sodium diminished strikingly, although the amount excreted in the urine, indicated by the figures below the columns, was relatively small. That the drop of sodium was not referable to excretion, but dilution, is more convincingly proved by the fact that its concentration returned to normal when the sugar had been burned, although

the excretion of salt increased in the second period. Such an increase of effective osmotic pressure should have an antidiuretic effect and doubtless would eventually have such an effect if the subject were not allowed any water. An indication of such an antidiuretic effect is evident if the first experiment on a relatively dehydrated subject is compared with the second on a well-hydrated subject. Although the amount of glucose given in the second was less and the rise of osmotic pressure smaller than in the first, the excretion of water and salt was far greater. Since an increase of effective osmotic pressure is the most compelling impulse to thirst, polydypsia ordinarily develops, giving rise to profuse diuresis. Since sodium chloride is the substance that normally constitutes almost the whole of the effective osmotic pressure, the normal means of correcting an increase of effective osmotic pressure is to accelerate the excretion of salt while retarding the excretion of water. This reaction appears to be used as a defense against hypertonicity even when this is due to components other than sodium chloride, such as glucose. In these experiments the excretions of sodium and chloride increased while blood glucose was elevated, although their concentrations in the serum were reduced, and fell again when the glucose had been consumed, although their concentrations in the serum rose. Sodium chloride may be regarded as the instrument by which the effective osmotic pressure of the serum is regulated. The broken lines on the columns represent the concentration of chloride. You will note that the changes of chloride and sodium in the

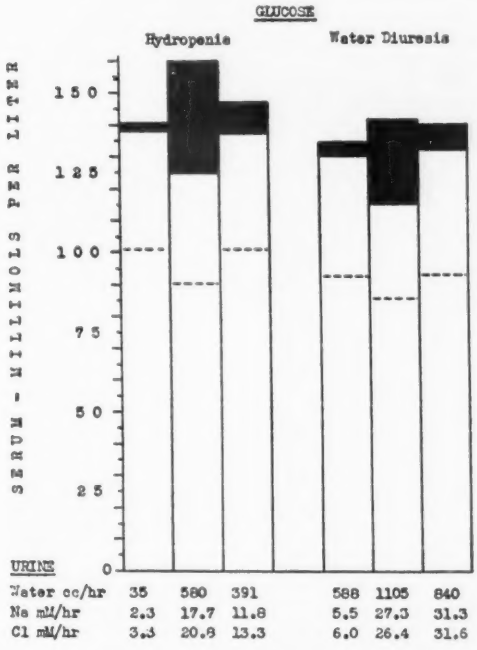

FIG. 2. The effect of the intravenous injection of hypertonic glucose solution on the serum and urine.

serum and their excretions in the urine are very similar. Hyperglycemia does, therefore, induce absolute depletion of sodium and chloride in the body; however, so long as hyperglycemia persists, the magnitude of the depletion is not so great as the depressed concentrations of sodium and chloride in the serum indicate.

In addition, the extreme diuretic effect of the glucose causes dehydration. Because of the hyperglycemia the cells participate in this in the manner described above. The extracellular fluid, however, bears the greatest brunt of the dehydration. If the hyperglycemia is eliminated without provision of sufficient water, the cells will withdraw water from the extracellular fluid, restoring their own hydration, but exaggerating the dehydration of the

Table 1. Water Content of Lactescent Serum

Case	*Serum*	*Water*	*Sodium*	*Cholesterol*		*Lipid P*	*Neutral Fat*
				Total	*Free*		
		%	*mEq./L*	*mg. %*	*mg. %*	*mg. %*	*mEq./L*
B82609	Original	72.7	102.5	....	...	...	....
	Bottom	90.2	135.0				
	Original	72.5	103.0	1219	581	53.0	478.0
	Bottom	91.3	134.5	115	...	...	....
	Original	78.0	115.5	1508	683	50.0	361.0
	Bottom	93.7	135.9	200	95	11.2	21.6
39-38-38	Original	...	120.0	1045	271	30.0	149.6
	Bottom	93.2	140.2	280	...	17.0	26.7

extracellular fluid. Vomiting accelerates the dehydration, and water given by mouth, while vomiting continues, enhances the dehydration, at the same time removing salt in excess of water.

The argument must be diverted for a moment to mention another feature that may confuse the interpretation of concentrations of electrolytes in the serum in diabetes. Recently a patient was admitted to the hospital with diabetic xanthomatosis and extreme hyperlipemia with lactescent serum. Although he did not seem extremely ill, had only moderate hyperglycemia and slight reduction of CO_2, the sodium and chloride of the serum were 102.5 and 74.5 milliequivalents per liter respectively. When the serum was centrifuged at 18,000 r.p.m., a speed at which the proteins are not layered, an extremely thick layer of fat collected at the surface of the serum. Analysis of the subnatant clear fluid revealed sodium 135.0 and chloride 102 mEq. per liter. The serum proved to have less than 80 per cent of water instead of the usual 92 per cent. The low concentrations of electrolytes

observed in the whole serum were due merely to displacement of water by insoluble lipids. This is illustrated in Table 1. It will be noted that although the concentration of sodium in the whole serum rose as the hyperlipemia diminished and the water of serum increased, its concentration in the subnatant fluid, marked "bottom," remained approximately constant and normal throughout.[1] The same was true of the concentrations of other electrolytes. Obviously when serum lipids—or at least insoluble lipids—are high, concentrations of electrolytes in serum require correction. The same phenomenon is illustrated in the last serum from a child with nephrosis. This may explain the puzzling low values for sodium encountered in some patients with this condition. It may be necessary to incorporate as part of accurate serum analyses measurements of water. Hyperlipemia and lactescence are common in diabetic acidosis. Reduction of the concentration of electrolytes in the serum in this condition may, therefore, be partly specious.

The sequence of events in the development of diabetic acidosis has been presented in this manner, quite apart from ketosis, to emphasize dehydration and salt loss, the chief causes of the shock and coma that characterize the condition. The major portion of the reduction of bicarbonate is the product merely of the replacement of this anion by ketone acids. To this extent it can and will be reconstituted as soon as the excessive production of these acids is abolished and the ketone bodies that have accumulated in the body have been burned. These two ends will be rapidly accomplished when the ability to burn carbohydrate is restored. The sodium combined with ketone acids is potentially bicarbonate. For therapeutic purposes in diabetic acidosis the difference between sodium and chloride is a more useful measure of bicarbonate than is bicarbonate itself. It is doubtful whether bicarbonate is ever urgently required; certainly it is not indicated if the bicarbonate plus potential bicarbonate is 30 mEq. or more. Under these circumstances it can only distort, not correct, the electrolyte pattern after ketosis is eliminated, by providing an excess of sodium. Administration of sodium lactate seems even less rational. If the lactic acid were burned, administration of this compound would be equivalent to the administration of sodium bicarbonate. Since lactic acid is probably not burned, but is converted to glycogen and then to glucose in severe acidosis, it is equivalent to sodium bicarbonate and glucose. Yet some who shudder at the use of glucose give lactate with complacency. Some sodium previously combined with bicarbonate is, to be sure, excreted with ketones in the urine before ammonia takes over the burden. However, this is not great and the kidneys, if given a reasonable chance to function, are able to adjust differences between ions with great facility.

In Figure 3 is shown graphically the initial status of 9 patients with diabetic acidosis described by Seldin and Tarail.[9] The first column depicts the serum of the average normal adult. The total height of each column represents the total osmolar concentration of those elements of the effective osmotic pressure that vary significantly. The black segments at the top are

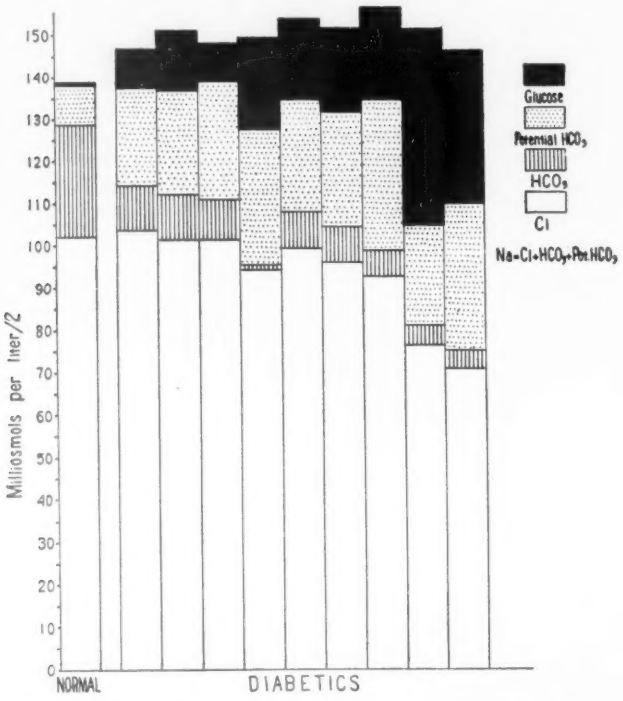

FIG. 3. The glucose and electrolytes of serum in patients with diabetic acidosis before treatment.

glucose; the remainder of each column is the concentration of sodium. On this are superimposed from below upward, chloride in white, bicarbonate in vertical lines, and the undetermined anions, in this case chiefly ketone bodies and therefore potential bicarbonate, stippled. The first and most striking feature is the enormous size of the glucose component. Actually the effective osmotic pressure of the serum is not diminished, as the concentration of sodium would indicate, but is increased. The cells are, therefore, desperately dehydrated, and the extracellular volume, even in its

depleted state, is sustained chiefly by water stolen from the cells. If this glucose were instantly burned, the amount of water withdrawn by the cells from the extracellular fluid might be disastrous. Second, comparison of sodium and chloride shows that they have been approximately equally affected by the dilution. Comparison of the bicarbonate and potential bicarbonate columns illustrates the point made earlier, that the reduction of bicarbonate is referable chiefly to replacement of this anion by ketone acids. The sum of existing bicarbonate and potential bicarbonate is not seriously reduced even in the patient who had only 1.3 mEq. of bicarbonate in the serum.

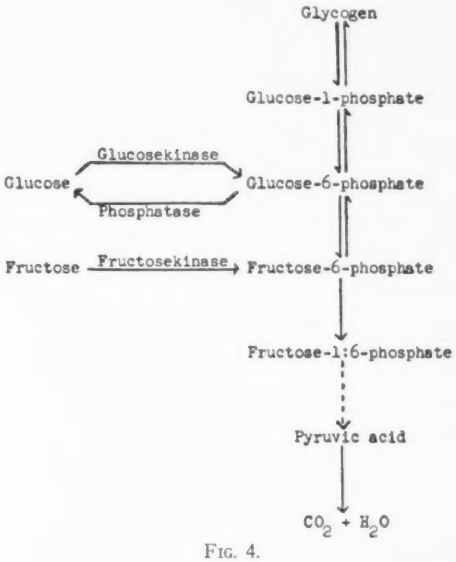

FIG. 4.

From the standpoint of carbohydrate metabolism, fat metabolism, ketosis, and destruction of protein, the first desideratum is to restore as rapidly as possible the ability to oxidize carbohydrates. The most obvious and the only undisputed measure for this purpose is the administration of generous amounts of insulin. It has been the impression of other competent observers and myself that frequent moderate doses, 50 units or less, are more effective than the same amount of insulin given in larger doses at longer intervals. The most important general guide is the blood sugar, evaluated in relation to the amount of sugar given.

The propriety, desirability, or necessity of giving glucose has been regarded by many as an all-or-none question, to be considered not on the basis of physiological knowledge, but of emotional inclinations. There can be no doubt that it facilitates the combustion of carbohydrate; to this extent hyperglycemia may be regarded as a protective or adaptive reaction. On the other hand, glucose and the hyperglycemia it induces or enhances promote polyuria, loss of salt, and dehydration—undesirable features. To meet this objection larger amounts of salt might be given. Although the

concentration of sodium in the serum is low, this is not a true measure of sodium depletion for the reasons given above. There is actually, in most cases, a greater deficiency of water than of salt. If, then, only saline solution is given to overcome the dehydration, when the hyperglycemia is abolished and the cells take back their water, the concentration of sodium in the serum will rise above normal. With such treatment, for example in the

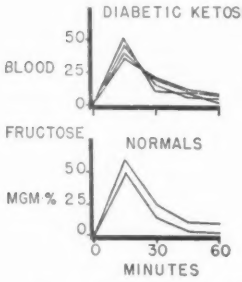

FIG. 5. The effects of intravenous injection of fructose in four patients with severe diabetic ketosis and two normal subjects. 100 cc. of 25 per cent fructose solution were injected intravenously from 0 to 10 minutes. In the diabetic patients, treatment with regular insulin and intravenous saline had been started prior to 0 minutes.

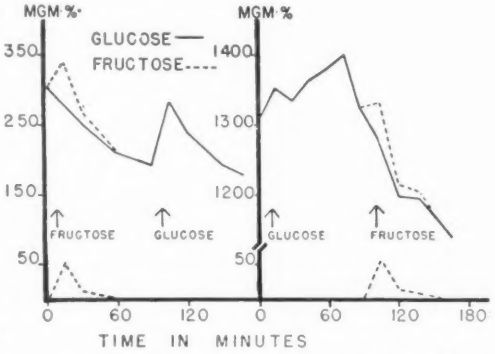

FIG. 6. The effects of intravenous injection of fructose and glucose in two patients with severe diabetic ketosis. Blood glucose is illustrated by solid lines; blood fructose by dotted lines. Blood fructose plus blood glucose equals total hexose. 100 cc. of a 25 per cent solution of fructose or glucose were injected intravenously in 10 minutes at the times indicated.

series of Danowski and associates,[8] values for sodium as high as 155 mEq. per liter were encountered. This means that the dehydration of the cells is prolonged, which should not be conducive to optimum metabolic activities. A certain amount of water in excess of salt is desirable. To provide this, the administration of either a sugar solution or a hypotonic salt solution is necessary. The latter, advocated by Butler,[3] if the hypotonicity is tempered, is a rational and safe procedure, but one that runs counter to conventions.

It has been demonstrated that this dilemma can be escaped by the substitution of fructose for glucose (Figure 4). The cellular metabolism of glucose, and perhaps its entrance into cells, is effected by an enzyme, glucosekinase, that requires the presence of insulin for its activation. The utilization of fructose, on the other hand, is initiated, at least in the liver, by a separate enzyme, fructosekinase, which does not require the intervention of insulin. Fructose, moreover, enters the oxidative pathway at a more

advanced point than glucose does. Finally, from the liver glucose can be returned directly to the blood by phosphatase, whereas fructose can escape only by the circuitous reverse path of conversion to glucose. Fructose is therefore removed from the blood of the patient with diabetic acidosis as rapidly as it is from the blood of a normal person. This is illustrated in Figure 5 from the paper of Darragh *et al.*[4] Moreover, perhaps because of the advanced point at which it enters the chain of oxidative reactions, a certain proportion of it can be utilized. Although it may contribute to blood glucose, if given rapidly, moderate amounts produce little or no hyperglycemia. This is illustrated in Figure 6 from the same paper. Fructose solution is, therefore, an ideal vehicle for the parenteral administration of water

TABLE 2. SERUM BICARBONATE—MEQ. PER LITER

		Minutes		
		0	*120*	*240*
NORMALS				
Acetate	(11)	25.2	30.5	28.8
Bicarbonate	(6)	25.6	31.3	29.8
DIABETICS				
Acetate	(8)	24.7	29.5	27.7
Bicarbonate	(6)	24.2	29.1	28.3

in diabetic acidosis and at the same time provides some utilizable sugar. It must be administered parenterally because it is largely converted to glucose in the process of absorption from the gut. We have used for some time a mixture of equal parts of 5% fructose and normal saline. We do not presume to claim that these are ideal proportions, but they have proved generally satisfactory. When the blood sugar approaches normal under the influence of insulin, glucose should be substituted for fructose to avoid hypoglycemia.

Fructose is also preferable to glucose as a parenteral sugar for diabetics without acidosis. We recently maintained such a patient for many days by a continuous infusion of fructose, providing all the carbohydrate required to spare protein wastage and prevent ketosis, without provoking hyperglycemia or glycosuria and without insulin.

Some doubt was expressed earlier in this discussion about the necessity for giving sodium bicarbonate in diabetic acidosis since the potential bicarbonate (sodium not combined with chloride) is relatively well preserved in this condition. Bicarbonate has been advocated for another reason. There is some evidence that it may accelerate the utilization of carbohydrate. Para-

doxically enough, it has also been found that alkalosis provokes ketosis. Although the central position of 2-carbon groups in the intermediary metabolism of carbohydrate and fat had been postulated by various investigators for a long time and the utilization of acetate had been demonstrated, participation of such 2-carbon groups in normal metabolism was not generally accepted because it was impossible to detect their presence in appreciable quantities in blood or tissues. Now that their participation has been unequivocally established it is evident that these acetyl groups, like ammonia, are so chemically or biologically active that, although they are being continually formed, they are as rapidly removed. There is then no acetyl pool as there is no ammonia pool.

TABLE 3. BLOOD SUGAR—MG. PER CENT

		Minutes			
		0	*120*	*180*	*240*
NORMALS					
Acetate	(9)	73	73	77	78
Bicarbonate	(6)	82	84	83	82
DIABETICS					
Acetate	(10)	217	196	191	187
Bicarbonate	(6)	215	185	183	181

Nevertheless, Lipsky and associates[5] attempted to ascertain whether intravenous administration of large quantities of sodium acetate would divert the metabolism into any particular channels. Twenty mg. of the salt were injected rapidly into normal subjects, patients with mild diabetes who did not require insulin, and patients with more severe diabetes from whom insulin was withheld during the day of the injection. The results can be only briefly capitulated. So far as the acid-base and electrolyte equilibria were concerned, the acetate was so rapidly removed that it had precisely the same effect as an equivalent amount of sodium bicarbonate on the sodium, CO_2 and Cl of the serum (Table 2). The acetate injections were, therefore, paralleled by injections of equivalent quantities of bicarbonate. The identity of the reactions to acetate and bicarbonate was just as evident in the intermediary metabolic reactions as it was in the electrolyte equilibria. Neither had any appreciable effect on the blood sugar of the normal individuals nor the mild diabetics with normal blood sugars (Table 3). Both definitely depressed the blood sugars of the severe diabetics with initial hyperglycemia, suggesting a beneficial effect on carbohydrate utilization. Both

increased the ketonemia of normal and mildly diabetic subjects slightly, but significantly. On the other hand, they increased the ketonemia of the more severe diabetics quite appreciably (Table 4). Reasons for the absence of any apparent specific effect of the acetate ion there is not time to discuss. It is, however, hard to build up from the experiments any very cogent argument for the administration of alkali in the treatment of diabetic acidosis, in the early stages at least, in behalf of the metabolic disorders.

It has been stated that blood sugar is the best running guide for the conduct of treatment. Urine sugar is unsatisfactory because collection of urine from patients in diabetic acidosis is too uncertain and analysis of

TABLE 4. BLOOD KETONE BODIES—MG. PER CENT

		Minutes			
		0	*120*	*180*	*240*
NORMALS					
Acetate	(8)	1.8	2.0	2.5	3.2
Bicarbonate	(6)	1.8	1.6	1.6	1.4
MILD DIABETICS					
Acetate	(5)	2.0	2.8	3.5	3.5
Bicarbonate	(4)	3.1	2.6	4.3	3.8
SEVERE DIABETICS					
Acetate	(4)	19.3	26.2	29.0	30.7
Bicarbonate	(2)	29.3	32.5	35.9	37.6

urine gives no information about the immediate status. The introduction of an inlying catheter with the possibility of infection is unjustifiable even with antibiotics available.

Since nausea and vomiting, outstanding features of diabetic acidosis, can only aggravate dehydration and salt depletion, nothing should be given by mouth until the patient is conscious and has been free from nausea and vomiting for some time. There is no reason to resort to tubes or to empty the stomach. The saline contents of the gastro-intestinal tract are as useful as any other fluids.

No other disorder can in such a brief period cause a more profound state of dehydration. Circulatory collapse is an ever-present danger. The patient with diabetic acidosis therefore should be grouped at once for transfusion, and blood should be ready for immediate use if required. If initial collapse does not respond rapidly to administration of fluids, transfusion should not be delayed. The administration of fluids should, however, be continued

simultaneously. The object of transfusion is to expand the volume of the circulating blood, not only by the increment of blood injected, but also by so improving the state of the circulation that it will pick up fluid from the tissues. This latter object is defeated if the extracellular fluid is depleted. Attention has been called to the necessity of providing carbohydrate more rapidly when the blood sugar concentration diminishes. The rate of administration of insulin should be retarded at the same time. A hypoglycemic reaction during recovery is hazardous.

Early in the development of acidosis the excretion of potassium and phosphate increases, though their concentrations in the serum do not fall. This discharge which clearly emanates from the cells and is out of proportion to the destruction of protein is probably connected with the impairment of carbohydrate combustion. As the condition advances and renal function suffers, the excretion of potassium and phosphate diminishes and their concentrations in the serum rise. In severe acidosis they are, therefore, more often high than low. As hydration is restored, their concentrations are reduced by the processes of dilution and accelerated excretion. When carbohydrate combustion is resumed, the cells begin to take up both potassium and phosphate, especially the latter, with such avidity that the serum may contain only traces of phosphate, and potassium may fall to 2 mEq. per liter or less. The hypopotassemia may give rise to serious symptoms, of which the chief are extreme weakness, collapse, and heart failure. The frequency and danger of this condition have, I believe, been exaggerated and many claims for miraculous recovery after injections of potassium are not justified by the evidence. There can be no doubt, however, that hypokalemia constitutes a distinct hazard and should be prevented by injection of potassium salts. Although frequent measurements of blood sugar are the best index of the success of treatment, they should be interspersed with measurements of bicarbonate, chloride, sodium, and potassium. Sole reliance for evaluation of potassium should not be placed on the electrocardiogram because this is not specific and because cardiac disorders should be anticipated and prevented. With modern methods an electrolyte analysis can be completed in an hour or less. If the serum potassium falls below 3.5 mEq. per liter, injection of potassium is indicated; 90 mEq. is ordinarily a satisfactory dose, though it may be necessary to repeat this. It should be given highly diluted in glucose or saline at a slow rate, with the blood flowing freely through the vein, since, if the potassium reaches a high concentration in the vein, it causes excruciating pain. Since phosphate is also greatly depleted, the use of potassium phosphate instead of the chloride appeals to the chemical esthetic sense, although the deficiency of phosphate

appears to have no deleterious effects. As soon as the patient can take a mixed diet, danger of hypokalemia is eliminated.

A certain number of patients will continue to die with diabetic acidosis as a result of the infection or other complication which precipitated the acidosis or because treatment is delayed until the subject is in a state of irreversible shock. Mortality can be reduced if the latest knowledge and technical facilities are utilized. The principles of prevention and therapy have been outlined. They should not, however, be reduced to a stereotyped routine. Details of their application must be individualized. Diabetic acidosis is an urgent state and its treatment must be intelligently and energetically conducted.

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AURICULAR EXCITABILITY OF THE NORMAL DOG HEART*

Experiments heretofore designed to examine the response of the heart to electrical stimulation were in general carried out under conditions which did not allow the heart to function in its normal environment. Most experiments were performed on isolated or open chest preparations in which factors of heat loss, CO_2 escape, and evaporation were difficult to control. Furthermore, the hearts were not usually allowed to beat spontaneously but instead were driven by an external stimulus.[13] In 1952 Sikand, Nahum, Levine, and Geller[14] by means of a cylindrical electrode passed into the heart through the chest wall were able to establish the excitability cycle of the dog ventricle in its normal physiological environment. Sufficient differences in the ventricular response of such a heart to electrical stimulation from previously reported studies made it desirable to determine whether the auricle in its normal state, like the ventricle, might exhibit an excitability curve different from that previously described.[4] The method used by Sikand *et al.* to study the problem in the ventricle was not applicable in the case of the auricle since it is not accessible to blind insertion of the cylindrical electrode. Accordingly, the electrodes were prepared by the method of Delgado and Sikand[5] and implanted aseptically onto each auricle by a slight modification described elsewhere.[12]

METHOD

Experiments were carried out on 18 dogs. The electrode attached to one auricle was used for bipolar stimulation and the electrode attached to the other auricle was used for visualizing the extrasystolic response. The electrogram, however, was obtained from the auricle whose excitability was being tested.

The method of stimulation and recording has already been described.[14] The excitability curve was determined by scanning the cardiac cycle at 5-15 msec. intervals. The excitability threshold was reached when an auricular extrasystole was visualized on the dual-beam oscillograph. One animal was tested by a 1 millisecond pulse, the remainder by 0.5 millisecond pulse. In most cases, complete electrocardiographic tracings were taken before, during, and after the experiment on a Sanborn Twin-Viso

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Cardiette. To prevent spontaneous changes in threshold, anesthesia and heart rate were held as stable as possible and stimuli were applied only every two minutes, since it was observed in the studies on the ventricle that the myocardium did not fully recover from the shock before the lapse of two minutes.[14] Most of the animals were tested on the day of implantation and on successive days until a threshold became unobtainable because it exceeded the limit of our generator.

RESULTS

In 14 animals the excitability cycle was determined two to four hours after the electrodes were implanted, the chest closed without a pneumo-

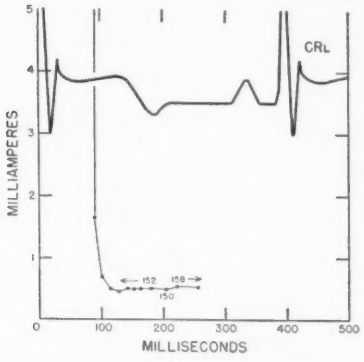

FIG. 1

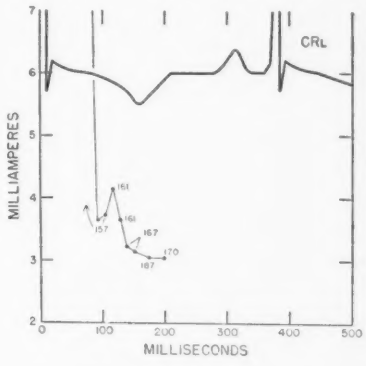

FIG. 2

FIG. 1. Excitability cycle obtained six hours after chest closure. Upper curve is precordial electrocardiogram taken with proximal electrode over left apex and distal electrode on right arm. Lower curve is excitability curve showing heart rates at different points on the curve.

FIG. 2. Excitability curve obtained 48 hours after electrode implantation. Upper curve is left precordial electrocardiogram, lower curve is excitability cycle. Note elevated diastolic threshold which begins at point labelled 187. Note the oscillation in relative refractory period. Numbers on curve represent heart rates.

thorax, and the animals were breathing spontaneously. It had previously been established that it requires about two hours for re-establishment of normal temperature conditions in the chest after it had been opened. In three of these animals the excitability curve was redone within two hours after the first determination, and in each instance the curves were identical in all particulars. In seven animals the excitability cycle was established first for the right auricle and then for the left. Some variability was encountered in the threshold level as well as in the duration of absolute and relative refractory periods. No specific threshold level could be identified as being characteristic of either right or left auricle. In four animals the

TABLE 1.

Animal No.	*Auricle*	*Exp. No.*	*Days post-op.*	*Heart rate per min.*	*Cycle ln. in ms.*	*TRP in ms.*	*ARP in ms.*	*RRP in ms.*	*TRP/RR in %*	*ARP/RR in %*	*RRP/RR in %*	*Diastolic threshold in mA.*	*Supernormal values in mA.*	*Duration supernormal in ms.*	*Current of injury*
7	Lt.	1	0	100	600	194	160	34	32.4	26.7	5.5	0.6			A
	Rt.	1	0	120	500	185	169	16	37.0	33.8	3.2	0.6	†(0.1)	(67)	A
9	Lt.	1	0	192	316	146	124	22	46.2	39.2	7.0	1.0			A
	Rt.	1	0	174	345	168	148	20	48.7	42.9	5.8	0.4			P
10	Lt.	1	0	144	417	167	153	14	40.1	36.7	3.4	0.7	†(0.1)	(18)	P
	Lt.	2	1	146	410	182	137	45	44.4	33.4	11.1	1.5			P
	Rt.	1	0	144	417	189	153	28	43.4	36.7	6.7	0.4			A
	Rt.	2	1	160	375	150	120	30	40.0	32.0	8.0	1.6			A
	Rt.	3	2	148	405	216	176	40	53.3	43.4	9.9	1.5			A
11	Lt.	1	0	177	339	147	124	23	43.4	36.6	6.8	1.72			
	Rt.	1	0	177	339	158	136	22	46.6	40.2	6.4	0.5			
	Rt.	2	1	158	380	136	122	14	35.8	32.1	3.7	1.5			
12	Lt.	1	0	130	462	223	192	31	49.4	41.6	6.8	1.49			
	Rt.	1	0	130	462	223	192	31	49.4	41.6	6.8	0.5			
13	Lt.	1	0	143	420	185	150	35	44.1	35.7	8.4	0.3			
	Lt.	2	1	136	442	172	147	25	38.9	33.3	5.6	1.31	0.16	100	
	(*Rt.	2	1	168	356	185	92	93	50.9	25.7	26.0	3.03		-	)
14	Lt.	1	0	130	451	183	170	13	39.7	36.9	2.8	1.0			A
	Lt.	2	1	137	438	210	195	15	47.9	44.3	3.6	2.0			A
	Rt.	1	0	111	541	190	170	20	35.1	31.4	3.7	0.5			A
	Rt.	2	1	111	541	218	189	29	40.3	34.9	5.4	0.5	0.13	125	A
15	Lt.	1	0	152	394	199	144	55	50.5	36.3	13.9	0.5			A
	Lt.	2	1	154	388	217	166	51	56.0	42.8	13.2	1.1			A
	Lt.	3	2	100	600	197	140	57	33.0	23.3	9.7	1.33			
	Lt.	4	3	77	773	215	145	70	27.8	18.8	9.0	1.68			
	Rt.	1	0	152	394	180	155	25	45.7	39.3	6.4	0.5			P
	Rt.	2	1	154	388	180	165	15	46.4	42.5	3.9	1.3			A

TABLE 1. (CONTINUED)

Animal No.	Auricle	Exp. No.	Days post-op.	Heart rate per min.	Cycle ln. in ms.	TRP in ms.	ARP in ms.	RRP in ms.	TRP/RR in %	ARP/RR in %	RRP/RR in %	Diastolic threshold in mA.	Supernormal values in mA.	Duration supernormal in ms.	Current of injury
	*(Rt.	3	2	100	600	205	130	75	34.2	21.7	12.5	1.4			A)
	*(Rt.	4	3	77	773	286	143	143	37.0	18.5	18.5	3.0			A)
16	Rt.	1a	0	137	438	214	201	13	48.8	45.9	2.9	0.1	0.15	90	P
	Rt.	1b	0	116	518	246	231	15	47.5	44.6	2.9	0.1	†(0.06)	(78)	P
	Rt.	2	1	118	510	221	206	15	43.4	40.4	3.0	0.58	0.11	90	P
17	Lt.	1	0	110	545	231	197	34	42.4	36.1	6.3	0.46			P
	Lt.	2	1	126	480	161	143	18	33.6	29.8	3.8	0.7			P
18	Lt.	1a	0	145	414	159	145	14	38.5	35.0	3.5	0.4	0.11	90	
	Lt.	1b	0	145	414	159	145	14	38.5	35.0	3.5	0.4	†(0.06)	(90)	
	Lt.	2	1	120	500	169	151	18	33.8	30.2	3.6	0.45			
19	Rt.	1a	0	109	550	250	200	50	45.5	36.4	9.1	0.76			A
	Rt.	1b	0	115	510	234	204	30	45.9	40.0	5.9	0.85			A
	Rt.	2	1	117	513	273	183	90	55.2	35.7	19.5	0.9			A
21	Lt.	1	1	136	441	173	134	39	39.1	30.3	8.8	1.5	0.27	70	A
22	Rt.	1	v	115	522	159	136	23	30.5	26.1	4.4	0.75	†(0.07)	(140)	P
23	Rt.	1	1	143	420	184	148	36	43.8	35.3	8.5	0.6	0.11	88	A
24	Rt.	1	1	115	520	165	151	14	31.7	29.6	2.6	1.0	†(0.06)	(58)	A
25	Lt.	1	0	135	444	167	135	32	37.6	30.4	7.2	0.17	†(0.03)	(30)	P
	Lt.	2	1	146	410	124	99	25	30.2	24.2	6.0	0.19	†(0.03)	(40)	P
26	Lt.	1	0	197	305	104	84	20	34.1	27.5	6.6	0.84			P
	Lt.	2	1	152	395	148	141	7	37.5	35.7	1.8	0.85	0.23	100	P

TRP—Total Refractory Period.
ARP—Absolute Refractory Period.
RRP—Relative Refractory Period.
RR—Cycle Length.
* Abnormal Curve.

mA.—Milliamperes.
ms.—Milliseconds.
A—Absent.
P—Present.
† Not Significant supernormal.

excitability was determined on only one auricle. All 24 experiments showed an absolute refractory period, a relative refractory period with a smoothly declining slope without oscillations or "dips." All showed a constant diastolic threshold averaging about 0.6 mA. (Fig. 1). This same type of excitability cycle was observed in 21 other experiments performed 24, 48, and 72 hours after electrode implantation except that in these observations the diastolic threshold rose to within 2 mA. (Table 1). Of these 45 experiments, 23 were performed on the left auricle and 22 on the right. No special curve emerged as characteristic of either the right or left auricle. The auricle is thus different than the ventricle, since in the latter, variations in the excitability curve were found between fibers at the apex and fibers at the base. Other animals that were studied after a time lapse of one to four days post-operative showed more striking changes in excitability than those just mentioned, the diastolic threshold rose progressively until finally it exceeded 12 mA. The relative refractory period showed periods of hyper- and hypoexcitability as well as plateaus (Fig. 2). It was considered that the rise in threshold was caused by the increasing fibrinosis under the polyethylene plate that held the electrodes and that this caused too wide a gap between the electrodes and the myocardium. The development of oscillations in the relative refractory period paralleled the development of reactive myocarditis under the fibrinosis. The close association between an altered excitability curve and reactive myocarditis of the tissue studied made it necessary to draw the inference that the altered curves were due to the pathological state of the tissue.[12]

The excitability response of the auricle in the normal physiological state. The total refractory period varied with the auricular rate, its shortest duration 104 msec. at a heart rate of 197, the longest 773 msec. at a heart rate of 77. In those experiments where heart rates were less than 135 per minute, the ratio of the total refractory period to cycle length was 0.39 but at faster heart rates the ratio of total refractory period to cycle length increased to 0.43. Stated in another way a relatively greater proportion of the cycle length is taken up by recovery at fast rates than at slow rates. The same phenomenon has been described also in the ventricle where it has greater significance since the cardiac output depends upon ventricular filling, which is in part dependent upon the duration of diastole.

An absolute refractory period was demonstrated in each experiment. It terminated on the auricular T (Fig. 4). Generally, the absolute refractory period, like the total refractory period, shortened as the cycle length shortened and lengthened as the cycle length increased.

The duration of the relative refractory varied widely from 7 to 90 msec. It bore no relationship to the duration of the absolute refractory period, the total refractory period, or the cycle length (Table 1). The progressive, smoothly declining slope was present in all observations.

The threshold period began with the end of the relative refractory period in over 82% of the experiments. No subnormal periods were encountered.

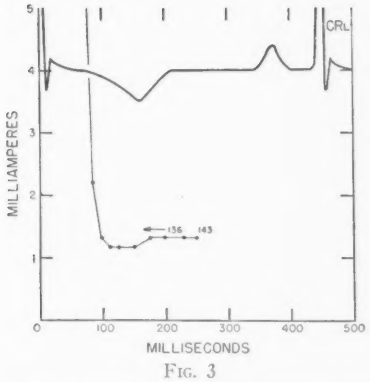

FIG. 3

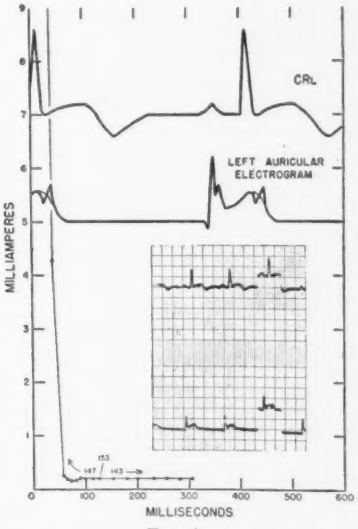

FIG. 4

FIG. 3. Excitability cycle obtained 12 hours after electrode implantation. Upper curve is left precordial electrocardiogram. Lower curve is excitability curve showing a shallow supernormal period. Numbers on curve represent heart rates.

FIG. 4. Simultaneous recording of (a) left precordial electrocardiogram upper drawing, (b) auricular electrogram middle drawing, and (c) excitability curve drawing with actual electrocardiogram in the insert. Note smooth diastolic threshold in excitability curve and injury current in ST segment of the auricular electrogram—in it the dotted line is our interpolation of the curve as altered by the superimposed ventricular component. Further description in text.

In each experiment the threshold level was constant, but in different animals the threshold varied from 0.3 to 1.0 mA. in 21 out of 23 determinations with an average 0.6 mA. In only two was a threshold level of 1.5 mA. recorded.

In 8 of the 45 experiments the relative refractory period was followed by a period of supernormal excitability from 70 to 125 msec. duration in which the auricles responded by an ectopic beat to a stimulus 0.1-0.17 mA.

below the diastolic threshold (Fig. 3). Three of these occurred on the day of implantation before pathology in the auricle developed and disappeared some days later, while the others appeared during the period of developing pathology.

The auricular electrogram was recorded in 33 experiments by leading from the stimulating electrodes. This made it possible to relate the electrical response with the excitability response of the same tissue. All electrograms showed a triphasic QRS, an ST segment, and a T wave. In 15 of the experiments a well-developed injury current was recognized by a sharp displacement of the ST segment which fused with the T wave (Fig. 4). In 18 experiments, however, no injury current was found. Of the 15 with an injury current, three exhibited a supernormal period, while three of the 18 experiments that did not show an injury current also showed a supernormal period. It was therefore not possible to relate supernormality with the electrocardiographic evidence of injury.

DISCUSSION

These experiments have permitted the establishment of a normal excitability curve of the dog auricle *in situ*. It was accomplished, however, by a method which required suture of an electrode plate to the auricle and prolonged trauma by the electrode points against the contracting auricle. It is clear that the curves obtained soon after implantation, before injury to the myocardium occurred, are the only ones that can reflect the excitability of normal myocardium. This was substantiated by histological studies several hours after implantation which revealed no fibrin on the epicardium and no myocarditis under the electrode points. The curves, however, obtained from one to seven days' postoperative varied both in diastolic threshold values as well as in the development of dips and oscillations in the relative refractory period. These latter curves, of which Figure 3 is an example, were associated with myocarditis and epicardial fibrinosis and were considered pathological curves.[12]

The normal curves of excitability were remarkably similar in that they all showed an absolute refractory period, a relative refractory period characterized by a smoothly declining slope, and a constant diastolic threshold level which averaged about 0.6 m.amp. None showed a subnormal period. In 17.8% a shallow supernormal period followed the relative refractory period. The absence of dips and oscillations in the relative refractory period of the normal auricle and their presence in an injured myocardium is a significant finding in these experiments. Since oscillations and "dips" in

the relative refractory period of auricles are believed to be involved in the mechanisms that induce fibrillation,[5] it becomes clear why normal auricles, which exhibit a relative refractory period without "dips," rarely show the phenomenon of fibrillation.

The duration of recovery in the normal auricle was shorter than in the ventricle.[14] This is in conformity with previous observations of other workers on exposed auricles.[3, 4, 6, 7, 11] Recent studies on cellular potentials of auricular and ventricular fibers of the dog heart have also shown that the duration of the potential curve of the auricular cell is shorter than that of the ventricular cell.[9] It will be seen below that the electrical component of excitation is identical with the duration of the total refractory period. The shorter refractory period of the auricle is probably due to the same factor that causes faster conductivity in auricular muscle than in the ventricle. Perhaps the size of the fibers is the basic cause since it is known that auricular fibers are larger than those of the ventricle.[10]

These experiments confirm earlier observations on the identity between the duration of the electrograms and the total refractory period. In each observation in which a bipolar electrogram was obtained from the muscle whose excitability was tested, the results showed the QT of the electrogram was of the same duration as the total refractory period. Mines[11] and also Adrian[1, 2] showed the same parallelism to hold for the exposed frog ventricle. The latter experimented on an abnormal preparation of an entirely different species at different temperature levels. Nevertheless, the duration of the electrogram and the refractory period was the same as it is in the dog. It is therefore probable that under any condition the duration of the electrical response of the auricle and ventricle is a fairly exact measure of the period of recovery from excitation. The observations previously reported on the normal dog ventricle are not exactly comparable with the experiments reported here on the auricle. In these experiments the correlation is between the electrogram of the auricular fibers being tested for excitability. In the studies on the normal ventricle the refractory period was compared with the precordial electrocardiograms which represented not the electrical response of the point tested, but of both ventricles.[14]

In addition to a shorter recovery period in the auricle than in the ventricle, the normal auricle also showed infrequent supernormal periods which were not observed at all in the excitability of the normal ventricle. Eight of the 45 physiological curves showed a supernormal period. In each instance it followed after the relative refractory period had been completed and therefore could not be confused with a period of hyperexcitability in the relative refractory period. The occurence of the supernormal period

could not be correlated with injury in the auricle, since it occurred as often in the absence of an injury current in the electrogram as in its presence. Neither could it be related to developing pathology which was known to occur, since the supernormal period disappeared in some animals in which pathological changes were developing, although it was present before such changes began. It could hardly be due to the anesthetic since all animals were anesthetized while only 17.8% showed a supernormal period. It may be significant in this regard that the ventricle under similar anesthesia did not exhibit a supernormal period. Since stimuli were applied only at two-minute intervals, the possibility of producing a supernormal period by too frequent test shocks was obviated.[4] However, the manipulations of the auricle by the technique of electrode implantation could not be altogether avoided as it was in the studies on ventricular excitability by the use of the cylindrical plunge electrode. Since a supernormal period has been produced experimentally by altering the normal physiological state in a variety of ways,[1,2,4,6,8] it is possible that the infrequent occurrence of a supernormal period may be due in some way to a change in the physiological state of the auricle caused by the manipulation or some sequela of it. It is therefore doubtful whether the supernormal period occasionally found can be considered a property of normal auricle.

SUMMARY

1. The excitability cycle of the intact dog auricle was established by using the method of permanent electrode implantation.

2. A curve of excitability of normal tissue determined soon after implantation showed an absolute refractory period, a relative refractory period of variable duration but with a smooth declining slope and a constant diastolic threshold.

3. The auricular electrograms showed a QRS, ST segment, and T wave.

4. The duration of the auricular electrogram was the same as the duration of the total refractory period.

5. Injury currents in the auricular electrogram occurred in almost half the experiments.

6. A supernormal period in 18% of cases was found in the physiological curves at the beginning of the diastolic threshold. Its occurrence as a property of normal auricle is questionable.

7. A curve showing "dips" and oscillations in the relative refractory period is shown as an example of several obtained from a pathological auricle.

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A RAPID TECHNIQUE FOR CONCENTRATION OF URINARY CHORIONIC GONADOTROPHIN FOR PREGNANCY TESTING*

False negative reactions in pregnancy testing with frogs and toads can be avoided by concentrating the urinary chorionic gonadotrophin. If unconcentrated urines are used, the incidence of these reactions may be as high as 43.7 per cent[1] and 50 per cent.[8] Inaccuracy occurs most frequently during the late summer and early autumn months when many batrachians are relatively insensitive, and also when urinary chorionic gonadotrophin titers are low, in very early pregnancies, and in the latter half of gestation.

To be useful, a concentration technique must be simple to perform and provide adequate recovery of the hormone. Furthermore, the final concentration product must be non-toxic and must not produce false positive reactions.

CONCENTRATION TECHNIQUES

For many years chorionic gonadotrophin has been precipitated from pregnancy urine with alcohol and acetone. If long periods are allowed for precipitation, the recovery is satisfactory, but the concentrates are frequently toxic.

Adsorption techniques using alumina, charcoal, Lloyd's reagent, kaolin, and many other substances have been described. In 1940 Scott[6] reported a kaolin adsorption technique for routine pregnancy testing. Hobson[2] used the kaolin concentration technique in 37,200 pregnancy tests and obtained an accuracy of 99.8 per cent, employing the female *Xenopus laevis* as a test animal. Workers in this country have not been so successful. Rheinhart, Caplan, and Shinowara[4] reported 76 per cent and 87 per cent accuracy. Samson[5] felt that the recovery of the hormone was about 50 per cent and discarded kaolin adsorption in favor of acetone precipitation. In our hands kaolin gives a non-toxic concentrate but only about 50 per cent recovery

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even though we have followed precisely the method outlined by Scott and modified by Hobson.

Because of the time required to carry out the kaolin adsorption technique and the relatively low recovery of hormone, a search was made for a more satisfactory adsorbent. Those studied include the cation exchange resins, Amberlite XE96, Amberlite IRC-50, Nalcite HCR, and Permutit H-70; anion exchange resins, Duolite A-7, A 40, Nalcite SAR, SBR, WBR, Permutit S-30, De-Acidite, and Amberlite IR-45. Other adsorbents used include aluminum oxide, silica gel, activated alumina, silicic acid, celite 545, hyflo super-cel, aluminum silicate, Fuller's earth, attapulgus clay, and

TABLE 1. EFFECT OF URINE pH ON THE RECOVERY OF CHORIONIC GONADOTROPHIN

Animal	*No. of animals*	*pH of urine*	*Positives*	*Negatives*
Male *Rana pipiens*	10	3.0	5	5
	10	3.5	5	5
	10	4.0	5	5
	10	4.5	6	4
	10	5.0	5	5

"Decalso." Unfortunately, the majority of these were unsatisfactory either at the adsorption or elution step. Work is still in progress on a number of the more promising of these agents.

KAOLIN ADSORPTION TECHNIQUE

The kaolin technique described below is quite satisfactory if it is recognized that there is only about 50 per cent recovery of the hormone and that there are critical points in the procedure where maximum loss of the hormone may occur.

pH: The two most important factors in this technique are the pH of the urine from which the hormone is adsorbed and the pH of the eluant. Hobson[3] states that peak adsorption on kaolin takes place at pH 4.0.

Our experience with experimental recoveries at pH 3.0 to pH 5.0 are given in Table 1. Standard chorionic gonadotrophin* was added to a large volume of urine. Five separate aliquots containing sufficient hormone to produce 50 per cent response were individually adjusted with 2.0 N hydro-

* Purified chorionic gonadotrophin supplied by Parke, Davis and Company. This was restandardized on male toads and frogs with the International Standard of chorionic gonadotrophin supplied by Mr. Adley B. Nichols of the Pharmacopoeial Convention, Inc., New York.

chloric acid and the hormone adsorbed on kaolin. The hormone was eluted with 0.1 N sodium hydroxide and the eluates injected into groups of 10 male *Rana pipiens* and the number of positive reactions noted. This very limited experiment suggests that this method of concentration is satisfactory over a urinary pH range from at least 3.0 to 5.0. For safety, the pH of the acidified urine should lie between pH 3.5 and 4.5; otherwise the recovery may be less than 50 per cent. Experimentation with known positive pregnancy urines also indicates that unless pH of the eluant is greater than 7.0 the hormone may not be recovered. Contact with solutions with a pH outside of the range of 3.0-11.0 causes partial destruction of the hormone,[7] the amount increasing with increased exposure time.

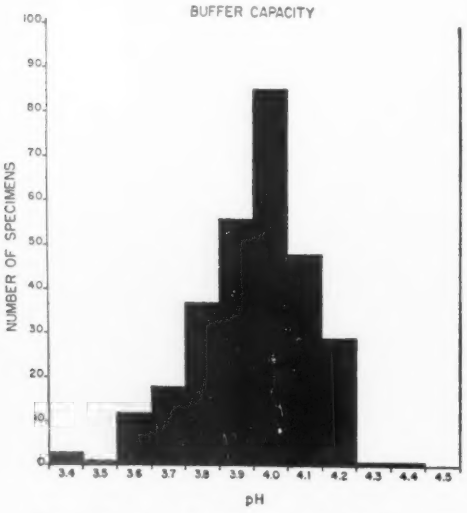

FIG. 1. The buffering effect of adding 5 volumes per cent of buffer to 287 consecutive urine specimens indicates that no specimens are outside the pH range 3.4 to 4.4. The majority fall between pH 3.9 and pH 4.1.

From the practical standpoint, most technicians find it difficult to achieve a relatively narrow pH range using indicator solutions, particularly when urines are bloody or highly colored. Although a pH meter may be used to adjust the pH, this procedure is time-consuming.

Buffer: It was therefore necessary to find a buffer which would keep urine within the desired pH range yet not be destructive to the hormone or toxic to the animal. An acetate buffer was finally developed that appeared to meet these requirements. Experimentally, urines were left at room temperature as long as one month. Before buffering, the pH of these urines reached about 9.0; with the addition of 5 ml. of buffer per 100 ml. of urine the samples were readily buffered into the range pH 3.5-4.5. The results obtained on 287 consecutive routine specimens submitted for the diagnosis of pregnancy are given in Figure 1.

To determine if this buffer destroyed the hormone, buffered urine containing hormone was allowed to stand three hours before adsorption was started. The eluate was tested in 40 male frogs and produced 22 positive and 18 negative reactions. When Scott's kaolin technique was employed with 40 control animals, there were 23 positive and 17 negative reactions. It was therefore concluded that the buffer does not destroy chorionic gonadotrophin.

Elution: Bromthymol blue indicator was added to the O.1 N sodium hydroxide eluting solution to warn the technician if the eluting solution was less than pH 7.0 as the result of acid adhering to the kaolin and urinary debris. When a buffer is used for acidification, it is necessary to introduce a washing step between the adsorption and elution steps to remove the residual buffering action.

Exposure of chorionic gonadotrophin to O.1 N sodium hydroxide for one hour did not cause detectable loss of activity, but about 70 per cent destruction was noted after three hours' exposure. Since the hormone is not exposed to O.1 N sodium hydroxide for more than a few minutes during the elution, no loss of hormone should occur during this step.

Separation: Various methods were studied for separating the kaolin from the acidified urine and eluant. In Scott's technique the kaolin was allowed to sediment from the acidified urine, the supernatant was either poured or suctioned off, and the remaining urine separated from the kaolin by centrifuging.

Separation can be accelerated by the use of a large centrifuge without waiting for the kaolin to settle out. Centrifuging is satisfactory but time-consuming. Moreover, washing the kaolin free of buffer is difficult, especially when large volumes of urine are used.

Separation of the adsorbents by filtration eliminates the time-consuming centrifugation steps and makes the washing step more efficient. Unfortunately, kaolin was found to block most filters capable of retaining it. However, the addition of calcined alumina of about 200 mesh to the kaolin suspension facilitates the filtration process without affecting recovery. Medium porosity sintered glass funnels provide rapid filtration and are capable of retaining the above adsorbents.

Our previous experience with calcined alumina and sodium hydroxide eluants indicated that it does not, of itself, produce false positive reactions in the male *Rana pipiens,* male *Bufo americanus americanus,* and male *Bufo marinus* animals.

MATERIALS AND METHODS

Apparatus: "Pyrex" 350 ml. medium porosity sintered glass funnel fitted with #6 rubber stopper through which a 1½" #15 gauge hypodermic needle has been inserted for suction.
Beaker clamp for holding funnel.
250 ml. wide mouth Erlenmeyer flasks.
Suction apparatus to which is attached rubber tubing with standard adapter to fit the hypodermic needle.
Rubber policeman.
#15 rubber stopper for use in cleaning the funnel.

Reagents:
1. Buffer
   Sodium acetate, 12.3 gm.
   Glacial acetic acid, 75.0 ml.
   Add 100.0 ml. of distilled water.
2. Kaolin-alumina suspension
   Washed and ignited kaolin, 20.0 gm.*
   Calcined alumina, 10.0 gm.**
   Add 100.0 ml. of distilled water.
3. Wash solution
   0.5 ml. of buffer (item "1" above) in 1,500 ml. of distilled water.
4. Sodium hydroxide-bromthymol blue eluting solution
   Sodium hydroxide, 0.1 N, 100 ml.
   Bromthymol blue 0.04 per cent solution, 5.0 ml.
5. Hydrochloric acid, 0.5 N
6. Sodium hydroxide, 0.5 N.

CONCENTRATION TECHNIQUE

The present technique consists of adsorption of urinary chorionic gonadotrophin on a kaolin-alumina suspension from urine buffered to about pH 4.0, separation and washing of the adsorbents on a sintered glass funnel, and elution of the hormone with 0.1 N sodium hydroxide-bromthymol blue solution.

Filtration method: The amount of urine to be concentrated depends on the sensitivity and species of the test animal and is roughly the same as used with Scott's kaolin technique. The urine is placed in a 250 ml. Erlenmeyer flask and 5 volumes per cent of the buffer and an equal volume of the kaolin-alumina suspension added. The buffered urine-kaolin-alumina suspension is shaken vigorously for about one minute, then poured rapidly into the sintered glass funnel to which a 250 ml. Erlenmeyer flask has been previously attached. Suction is then applied through the hypodermic needle. After the filtration has been completed for about 10-15 seconds, the Erlenmeyer flask which contained the buffered urine-kaolin-alumina suspension is rinsed with about 25 ml. of the wash solution (item #3), which is then poured over the adsorbents retained by the

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* J. T. Baker, Phillipsburg, Pa. Kaolin should be washed to remove very fine particles.

** Aluminum Company of America, Calcined Alumina A-3 remesh and material smaller than 200 mesh discarded.

funnel. A second wash with 25 ml. of the wash solution is then performed. This water wash is designed to remove excess buffer and thus allow the eluant to carry the suspension into the alkaline range. When this wash has cleared the filter, suction should be *disconnected*, the filtrate discarded, and a clean 250 ml. flask or small specimen bottle attached to the filter.

Ten ml. of 0.1 N sodium hydroxide-bromthymol blue solution is added to the precipitate which is then carefully mixed with the rubber policeman. It is very important to mix the reagents thoroughly; otherwise elution will not be complete. It is equally important that the suspension following mixing remains blue or bluish-green. If this is not the case, a few drops of 0.5 N sodium hydroxide are added until the desired color is obtained; otherwise the hormone will not be eluted.

If more than a drop or two of 0.5 N sodium hydroxide is required, the adsorbent has been inadequately washed. To avoid toxicity from the high electrolyte content which occurs in such eluates, the eluant, after neutralization with 0.5 N hydrochloric acid as described below, should be dialyzed for 15 to 20 minutes before injection.

The suspension is allowed to stand one minute or longer before re-connecting the suction. The eluant thus collected in the Erlenmeyer flask should be blue or bluish-green. Hydrochloric acid 0.5 N is added drop by drop until the fluid just turns yellow. The eluant is then ready for injection.

Centrifuge method: The appropriate amount of urine is placed in a 250 ml. centrifuge bottle and 5 volumes per cent of the buffer and an equal volume of the kaolin-alumina suspension added. The buffered urine-kaolin-alumina suspension is shaken vigorously for about one minute and then centrifuged for three minutes at 2,000 rpm.

Following centrifuging the supernatant fluid is discarded and about 100-150 ml. of distilled water added to the precipitate. The centrifuge bottle is vigorously shaken for one minute and centrifuged again as outlined previously. This water wash is designed to remove excess buffer and thus allow the eluant to carry the suspension into the alkaline range.

Add 10 ml. of 0.1 N sodium hydroxide-bromthymol blue solution to the precipitate and then follow the eluting procedure outlined previously under "Filtration Technique."

Centrifuge the suspension for three minutes at 2,000 rpm. and decant the blue-green supernatant fluid into a test tube. Hydrochloric acid 0.5 N is added drop by drop until the fluid just turns yellow. The total fluid is then drawn up into a 10 ml. syringe and half the total volume injected into each of two animals.

RECOVERY EXPERIMENTS

An attempt was made to compare directly recoveries obtained by the above methods and by Scott's technique. A known quantity of a laboratory standard of purified chorionic gonadotrophin was added to male urines, the amount of hormone added being determined by the sensitivity of the animals used. Because the expected recovery was about 50 per cent, double the amount of hormone required to produce just less than 100 per cent response by direct injection was added to urines before concentration. The hormone present in 150 ml. of a given specimen was concentrated with the filtration technique and the percentage of positive reactions thus obtained compared with the results of concentrating 150 ml. of the same specimen with Scott's technique. From Table 2 it will be seen that the percentage response is about the same for both tech-

niques. Statistical analysis indicates that such differences as were observed, or even greater differences, may have been expected to occur as a result of chance alone in more than 80 out of 100 trials ($0.8 < P < 0.9$).

It should be noted that recovery of chorionic gonadotrophin using kaolin adsorption varies slightly with the concentration of gonadotrophin present, there being less

TABLE 2. RECOVERY EXPERIMENTS. FILTRATION VS. SCOTT'S TECHNIQUE

Exp.	*Animal*	*C.G. added per animal*	*Filtration technique*		*Scott's technique*	
			No. of animals	*% Response*	*No. of animals*	*% Response*
1	*R. pipiens*	60 IU	30	93.3	23	95.6
2	*R. pipiens*	60 IU	30	83.3	15	86.7
3*	*R. pipiens*	40 IU	60	96.7	15	80.0
4	*B. marinus*	150 IU	15	86.7	27	96.3
			135		80	

* Animals more sensitive than those used in Exp. 1 and 2.

TABLE 3. RECOVERY VS. CONCENTRATION OF CHORIONIC GONADOTROPHIN

Animal	*C.G. added per animal*	*Concentration of C.G. per 100 ml.*	*Number of animals*	*% Response*
R. pipiens	60 IU	600 IU	60	88.3
R. pipiens	60 IU	80 IU	12	58.3
B. marinus	150 IU	1500 IU	15	86.7
B. marinus	150 IU	200 IU	24	62.5

recovery from dilute solutions. Table 3 illustrates this phenomenon. For example, with a concentration of 80 IU per 100 ml., 58.3 per cent response was obtained. This is contrasted with 88.3 per cent response when the concentration was 600 IU per 100 ml.

COMMENT

The centrifuge buffer kaolin technique and more recently the filtration technique have been used with a high degree of accuracy in over 1,100 pregnancy tests. These clinical results will be reported in detail elsewhere.

With the filtration technique a minimum of equipment is necessary and the running time on a 150 ml. specimen is eight to ten minutes. With a large centrifuge and buffer the comparable procedure takes 20 minutes, and 40 to 50 minutes are required if Hobson's modification of Scott's kaolin technique is employed. The above running times apply to single specimens;

if three to four specimens are run simultaneously, individual running times are shortened considerably. In the case of the filtration technique it is relatively easy to complete four concentration procedures in 20 minutes.

We have constructed a simple piece of apparatus which holds four funnels side by side, and have thereby reduced the amount of manipulation necessary. The funnel requires a minimum amount of care, but it should be thoroughly cleaned at the end of each procedure. Most of the kaolin-alumina can be removed with an ordinary bottle brush and running water. The #15 rubber stopper should then be placed in the mouth of the funnel and about 250-300 ml. of hot water back-suctioned through the filter to remove kaolin which may be deep in the filter pores.

While the final procedure is technically simple, accurate results will depend on careful initial checking of the reagents and filters. These precautions should be observed:

1. The buffer pH should be about 3.3 and the kaolin must be coarse.
2. With a new funnel, a trial experiment with a negative urine should be run before routine pregnancy testing is done to insure that the funnel porosity is fine enough to retain the adsorbents.
3. Whenever a new supply of reagents is made up, it is desirable to check them with a known positive pregnancy urine or with standard chorionic gonadotrophin.
4. If the urine is grossly bloody or contains large amounts of particulate matter, it should be filtered through coarse filter paper before the concentration procedure is started.

SUMMARY AND CONCLUSIONS

A simple, rapid, economical concentration technique for pregnancy testing has been described. Recovery is adequate, the concentrate is non-toxic, and no false positive reactions were noted in the animals studied.

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EXPERIMENTAL CORONARY OCCLUSION USING A POLYETHYLENE TUBE: A PRELIMINARY REPORT†

In addition to pathological and physiological studies of infarcts, many investigators are now studying methods for bringing additional arterial blood to the heart. Though frequently called "revascularization," as would be the case in human beings, most of the experiments are actually attempts at supervascularization because there is no inadequacy of circulation at the time the procedures are done. It would seem wiser to bring collateral circulation to a dog's heart having a severe deficiency at the time the collateral is to develop. The procedure can then be better evaluated later by a more physiological or direct method than by the statistics of survival after ligation of the anterior descending artery. A major difficulty in accomplishing this has been the high mortality associated with attempts to produce large infarcts in dogs.

Collateral circulation develops in the body in general when arterial supply is inadequate. Whereas capillaries develop in response to fibroplasia stimulated by many different factors,[1] differentiation into larger vessels is apparently dependent on the so-called "demand" by the tissue. Though the nature of this "demand" is not understood, a logical hypothesis is that it develops in response to abnormal pressure relationships.[14]

Many ingenious methods have been used in the past for producing infarcts in dogs, but unfortunately the statistics regarding the operative and late mortality and the extent of infarct obtained are incomplete in many of the reports. Beck and his co-workers[8] have well established the fact that infarcts are obtained in all dogs which survive ligation of the anterior descending artery directly at its origin. About half were larger than four centimeters and were full thickness infarcts. A minority were small and are subendocardial. However, the operative mortality is consistently 70%.

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Of the various methods used for producing a slow, chronic occlusion of the coronary arteries in the dog, constriction by irritant polyethylene[12] appears to have given the best results but is somewhat unreliable and produces an occlusion so slowly that inter-coronary collateral channels are able to develop fast enough to avoid much infarct. Another chronic method is the ring attached to the chest wall with linen thread which cuts through the artery after a period of time.[4] Insufficient details are given to evaluate the reliability of this method.

Methods for delayed acute occlusions in a dog which has recovered from surgery include the double carrick bend,[11] the spring clamp held open with catgut,[13] screw clamps,[2,9] and various types of snares. In some of these methods there is the disadvantage of a foreign body protruding through the chest wall which may be a problem in chronic experiments. There is some evidence[10] that an acute occlusion results in a higher mortality in a conscious than in an anesthetized dog. There would seem to be a place in experimental surgery for a technique which would consistently produce a large infarct with a low operative mortality.

The timing is critical because the dog's heart answers its own demand for collateral circulation by the rapid development of inter-coronary arterial anastomoses from other areas which have abundant arterial supply. In man, however, the myocardium may be abnormal and more diffusely anemic than is the case in the normal dog. The occlusion in the dog to be effective must be timed so that the collateral connections forming from the surgically produced extra-cardiac source may develop in competition with the natural collateral channels.

Insertion of a small piece of polyethylene tube into the anterior descending artery of dogs results in the formation of a clot over a period of about 24 hours, as may well be expected on the basis of the experience with polyethylene in other arteries in unheparinized dogs. In the present series of experiments this technique has consistently given infarcts with an encouragingly low mortality.

TECHNIQUE

Twenty-five normal dogs were anesthetized with veterinary nembutal and placed flat on the operating table with the left side up. The chest was opened by removing the left fifth rib using aseptic technique. Endotracheal respiration was maintained by 100% oxygen under intermittent positive pressure, using a minute breathing volume of one liter per minute per 10 pounds of body weight, and later adding one to two liters per minute just before the period of occlusion. This ventilation was usually enough to avoid any attempt on the part of the dog to breathe spontaneously and presumably means that hypercapnia was avoided.

The pericardium was opened and retracted with sutures. The first 2 to 2.5 cm. of the anterior descending artery were dissected free on all sides with the aid of 3-0 silks for retraction. The small branches were ligated, and a double loop of 3-0 silk placed around the large ones. It was found important to occlude all branches in order to prevent back bleeding during the cannulation. The ends of the dissected portion of the anterior descending artery were clamped with Potts ductus clamps and a transverse

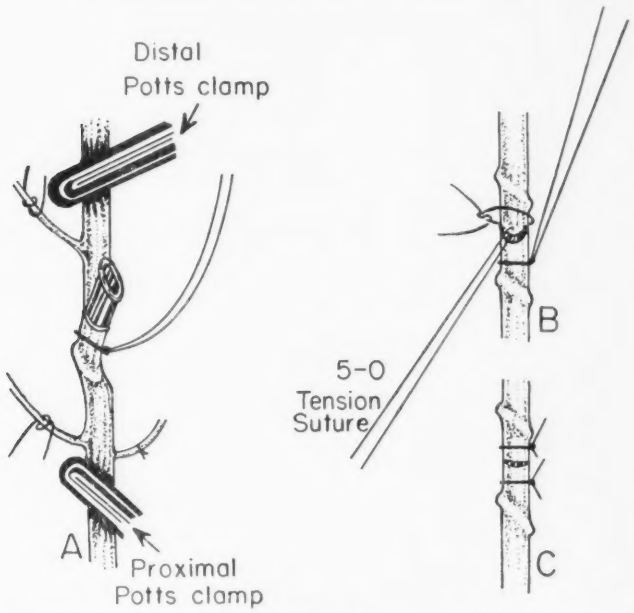

FIG. 1. Steps in the insertion of the polyethylene tubing. After the proximal silk is tied, it is used to pull the tube into the distal end of the artery which is pulled in the opposite direction by the 5-0 silk. The distal silk is then tied.

incision made in the center of the occluded area for about three-fourths of the circumference of the vessel. A 5-0 silk suture on an atraumatic needle was placed through the distal lip of the incised artery at this point. The proximal lip was then grasped with small tooth forceps and the tube inserted (Figs. 1 and 2). The previously placed 3-0 silk was tied around the vessel and the proximal end of the tube, and the end secured by a hemostat to be used in pulling the tube into the distal end of the vessel. By pulling distally on this silk and proximally on the 5-0 silk on the distal lip of the incision, the distal tie could be placed. The proximal Potts clamp was then removed, and it was found essential to punch a small hole in the artery just proximal to the distal Potts clamp and permit bleeding for several seconds to assure removal of any air remaining in the tube. After some practice, the procedure usually required that the artery be occluded for no longer than 4 to 8 minutes.

RESULTS

Of the twenty-five dogs subjected to this procedure, there were six operative deaths, all in the first ten. In three cases the ventricle fibrillated, either after the anterior descending artery had been occluded for longer than 12 minutes during the cannulation or just after release of the Potts clamps. The fibrillation in the latter circumstance was probably the result of inadequate removal of air before releasing the distal clamp, but could pos-

TABLE 1.

Operative deaths	6
Anomalously small artery	2
Died within 24 hours	2
Late deaths	2
Sacrificed	13
Total	25

TABLE 2. EXTENT OF INFARCTS IN 10 HEARTS SECTIONED MICROSCOPICALLY

	Full thickness infarcts	
Follow-up time	*Complete*	*Patchy*
5 days		1
2 weeks		2
5-6 weeks	1	3
3 months	3	

sibly be the same fibrillation noted by others following the release of a temporary occlusion.[3, 16] Either the attempt at defibrillation was unsuccessful in these dogs or they had suffered irreversible myocardial damage which caused death within the first several hours post-operative. Two other dogs were technically unsatisfactory because of an anomalous anterior descending artery too small to cannulate. Though both of these dogs survived ligation, the infarcts were small and they must be considered failures in the analysis.

One dog died the day after surgery immediately after being given a large dose of distemper antiserum. There were three dogs in which death resulting from the coronary occlusion cannot be ruled out. One dog died the night of operation, and autopsy showed the polyethylene tube less than 50 per cent occluded. Another dog died after five days and another after one week, probably of canine hepatitis though this diagnosis was not

FIG. 2. The tube used. A section of 1.57 mm. i.d. and 2.08 mm. o.d. polyethylene tubing is cut obliquely, and the ends flared by a gentle flame. Digits in scale are 1 millimeter apart, and total length of the tube is about 8 millimeters.

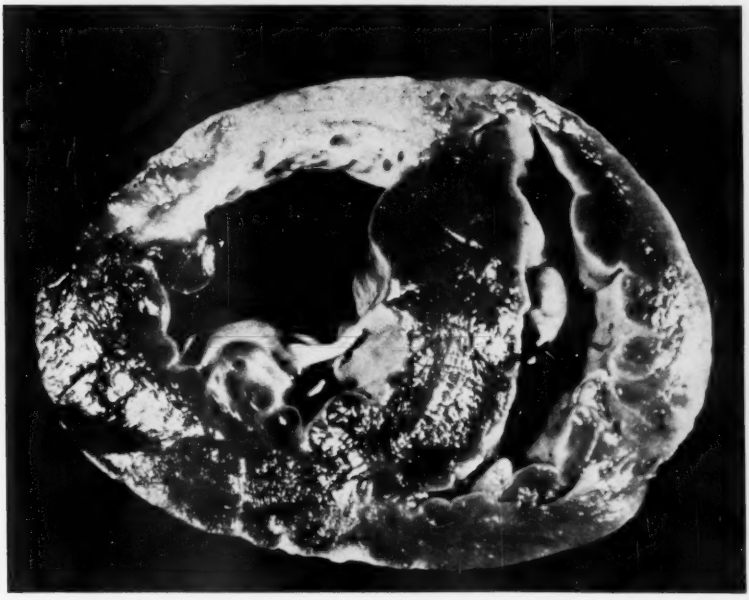

FIG. 3. Heart from a dog sacrificed after 3 months. The infarcted left ventricular anterior wall is in the upper left portion of the photograph.

FIG. 4. Section of the same heart, taken one centimeter toward the apex.

FIG. 5. The edge of an infarct from a dog sacrificed after 6 weeks showing fibrous areas and adjacent muscle. H and E stain. x 35.

proved. The remaining 13 dogs were sacrificed (Table 1) at times according to Table 2.

A total of 15 dogs were followed five days or longer, a period considered long enough to develop an infarct. Five of the hearts were inspected externally and then a vinylite corrosion cast was made of the coronary circulation. There was complete occlusion of the polyethylene in all cases. Two of these five showed infarcts visible externally and three did not.

Ten of the hearts were sectioned grossly and microscopically. All of them showed infarcts. Four had complete destruction of the muscle fibers through the full thickness of the ventricular wall (Figs. 3 and 4). Six had patchy infarcts, usually with most of the muscle gone in the inner half and the most of it present in the outer half (Table 2). The dogs with complete infarcts followed for longer than five weeks showed definite thinning of the ventricle and paradoxical motion on inspection of the thoracic contents just before sacrifice.

The infarcted area consisted microscopically of total destruction of the muscle fibers in most areas with fibrous replacement, though in some specimens there were patchy islands of muscle fibers remaining (Fig. 5). Some of the fibers appeared to be normal muscle while others showed swelling and loss of normal striations. The infarcts at the widest portion involved an average of one-fourth of the circumference of the circle formed by the left ventricle and the septum and in most cases actually involved the anterior portion of the septum and the proximal part of the right ventricular wall. The infarcts extended from within one cm. distal to the ligation almost to the apex of the hearts.

DISCUSSION

It appears that the mortality has been definitely decreased over that which would have been expected from acute ligation. Of the 17 dogs in which polyethylene was technically satisfactorily inserted, the early and late operative mortality attributable to the occlusion consisted of a maximum of three dogs and a minimum of 0, depending upon the actual cause of death in the three uncertain cases. The occlusion was always one centimeter or less from the origin, and in most cases was about 0.5 cm. However, the 70% mortality obtained by Beck *et al.* from ligation exactly at the origin is not comparable. The incidence of fibrillation from ligation in the first two centimeters of the anterior descending artery varies greatly in the literature. Manning reports 10%,[10] Carter, 48%,[5] Gross, 50%,[7] Harris, 54%,[9] Schauer, 67%,[15] Orias, 69%,[13] and Tennant, 71%.[16] In the first 10 dogs of this series during which the operative technique was being developed and the anterior

descending artery occluded for more than 12 minutes during the cannulation, 3 dogs fibrillated and 3 did not. These three would doubtless also have died had the artery been simply ligated.

Ten dogs in which all branches proximal to the polyethylene were definitely ligated at operation have been done since the 25 herein reported. The gross mortality consisted of two dogs, one of which was technically unsatisfactory and clotted prematurely due to angulation of the polyethylene, and the other was demonstrated to have distemper. The survivors have not been posted, but are expected to have as large or larger infarcts than those in the reported series.

The onset of significant occlusion by clotting in the polyethylene is believed to occur from three to six hours post-operatively as judged by beginning changes in the ECG, and the several specimens seen early post-operatively suggest that the occlusion is complete at about 24 to 30 hours. The mechanism of protection against ventricular fibrillation by this semi-gradual occlusion is not known. It is possible that vasomotor tone of the existing collateral channels is relaxed if the occlusion is gradual. Other mechanisms may be involved, such as avoidance of the high concentration of toxic products of muscle ischemia that would be obtained after acute ligation.

No drugs were used to decrease irritability of the heart but adequate quantities of oxygen were definitely assured.

SUMMARY

A technique for the insertion of a small piece of polyethylene tubing into the anterior descending coronary artery of the dog to produce occlusion as the polyethylene clots over a period of about 3 to 30 hours post-operatively has been described. There was no operative mortality after the technique was mastered, and the late mortality was much lower than would have been expected after acute ligation. Infarcts were present in all the hearts sectioned.

This technique may be useful for producing a demand for circulation by the heart with a reasonably low mortality, for stimulation of collateral circulation in procedures designed to increase the arterial blood supply to the heart.

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STUDIES ON THE INFECTION OF TUMOR CELLS BY EXTRANEOUS VIRUSES. I. ORNITHOSIS VIRUS INFECTION IN TUMOR-BEARING MICE**

The fact that viruses can multiply in tumors has been recognized since the original work of Levaditi and Nicolau[8] and is now known to pertain to many viruses,[13, 14] including those endowed with a strict tissue tropism such as rabies.[10, 16] However, these observations hold true for hosts which are not immune to the virus in question, whereas a virus injected into tumors of a virus-immune animal will fail to flourish. This was clearly shown by Levaditi and Nicolau,[9] who inoculated vaccine virus into a transplantable epithelioma growing in rats immunized against the virus and found that the virus failed to multiply. In this instance the tumor acquired the property of inactivating virus equivalent to that of other tissues such as skin, brain, and testicle.

However, when these "resistant" tumors were transplanted further to normal rats, immunity was lost, and the tumor was once again a fertile ground for the virus. Additional evidence against the "immune tumor" appears in the work of Rivers and Pearce[15] who infected the Brown-Pearce carcinoma of rabbits with virus III or vaccinia and found that the virus persisted longer in the tumor than it did in any other tissue. These observations support the hypothesis that the tumor is not capable of an efficient immunological response which is perhaps a unique character of the tumor cell.

From the evidence we must now admit to a paradoxical situation in which a virus, vaccinia, is still demonstrable in the tumor long after the host has developed an immunity against the virus, while, on the other hand, the virus never multiplied in the tumor when the latter was transplanted to an immune host. It is possible that this apparent conflict may be associated with differences in concentration of the viral antibody in the normal tissues and tumor; therefore an appraisal of these neutralizing antibodies was thought to be indispensable for a correct resolution of the problem.

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Since viruses of the psittacosis-lymphogranuloma group are known to elicit a poor antibody response,[7] they were particularly appropriate for this study. Accordingly, an ornithosis virus was selected for the experiments reported here and in the preliminary studies which have been published.[4]

MATERIALS AND METHODS

Virus. The KAM strain was used as the test virus which was made available to us through the courtesy of Dr. G. Rake of the Squibb Institute for Medical Research. This virus is highly pathogenic for mice by the intracerebral inoculation route, but after intraperitoneal inoculation, death will occur only when large amounts of virus are inoculated. This virus is resistant to sulfonamide and multiplies well in tissue culture media which contains 10^{-3}M sulfathiazole. Contrary to the classical psittacosis virus, the virus is pathogenic for pigeons by intracerebral route and is abundantly demonstrable in the meninges by impression smears. From these properties it would seem that the KAM strain belongs to the ornithosis group and may be identical to the so-called egg line of Hillemann.[3] The virus has been maintained in mice by serial intracerebral inoculation and is now in its 210th generation.

For inoculation purposes the pooled brain tissue from moribund mice was ground with sand and a 10% extract in saline obtained. The extract was centrifuged at 2,000 rpm. for 10 minutes and the supernate used for injection. At every experiment the potency of the supernate was titrated in the mouse brain.

Tumors. 1. *Quinone-induced carcinoma.*[18] This tumor was produced by daily cutaneous application of 0.2% parabenzoquinone to the skin of mice and, once the tumor was apparent, successive transplants to normal mice. Histologically the tumor is of spindle cell carcinoma and has been used in the present experiments between its 80th to 110th generation.

2. *Fructose-induced sarcoma.*[19] The tumor was produced in mice by daily subcutaneous injection of 25% solution of fructose over 300 days. Histologically it is a fibrosarcoma. Both of these tumors were prepared by Dr. Takizawa and his associates from the Department of Pathology and through their courtesy were made available to us. The tumors were passed through mice by trochar grafting of pieces about 2 mm. in diameter. Growth occurred in practically every case.

Estimation of virus infectivity. The tissues were ground with glass powder and enough saline to make a 10 per cent extract. After centrifuging these suspensions at 2,000 rpm. for 10 minutes, supernatants were diluted with beef infusion broth in tenfold serial dilution and 0.03 ml. of each dilution was inoculated into groups of four mice. The animals were observed for two weeks and the LD_{50} was calculated by the method of Reed and Muench.[18] Non-inbred albino mice weighing about 12 gm., purchased from the local dealer, were used throughout.

Virus neutralization test. A 20 per cent suspension of mouse brain infected with the ornithosis virus was diluted in tenfold serial dilutions, and to each dilution of virus an equal volume of pooled serum and carcinoma extract and liver extract was added. As controls we used normal serum, carcinoma and liver extracts from non-infected mice. Test and control mixtures were kept for 30 minutes at room temperature and then inoculated into groups of 5 mice intracerebrally.

EXPERIMENTAL

DISTRIBUTION OF THE ORNITHOSIS VIRUS IN MOUSE TISSUES AFTER INTRAPERITONEAL INOCULATION

Two groups of 20 mice each were injected in the peritoneum with 0.2 ml. of extract of mouse brain at either 10^{-1} or 10^{-3}. The LD_{50} of this virus suspension was $10^{-4.8}$ by intracerebral route. Two mice from both groups were sacrificed 2, 5, 8, 11, and 14 days after virus inoculation and the LD_{50} of their liver, spleen, lung, and blood —as obtained by cardiac puncture—were each estimated by intracerebral titration in mice.

The results as shown in Table 1 indicate that the virus which was injected intraperitoneally multiplied abundantly in the liver and spleen. In several mice, sticky

TABLE 1. TITERS OF ORNITHOSIS VIRUS IN TISSUES OF MICE AFTER INTRAPERITONEAL INOCULATION

	Dilutions of the inoculum							
	10^{-1}				10^{-3}			
Days after inoculation	*Liver*	*Spleen*	*Lung*	*Blood*	*Liver*	*Spleen*	*Lung*	*Blood*
2	2.5	2.8	1.0	1.5	1.0	1.8	<1.0	<1.0
5	4.8	4.0	3.0	1.5	4.3	4.0	2.8	1.0
8	4.8	4.0	2.0	<1.5	5.0	4.5	2.3	2.5
11	4.5	3.8	3.0	2.0	5.0	5.0	2.8	1.0
14	2.0	2.5	1.5	<1.0	1.5	2.0	1.8	<1.0

Figures are log of LD_{50}.

exudates were observed in the peritoneal cavity when the mice were sacrificed from 5-11 days after virus inoculation. The results are of interest since ornithosis virus is considered as being identical with meningo-encephalitis virus[12] which is characterized by a strong affinity for meninges and lungs.

AFFINITY OF THE ORNITHOSIS VIRUS FOR TUMORS

1. *Experiments with a carcinoma.* Eight mice bearing tumors nine days old were injected in the tumor with 0.2 cc. of a 10^{-1} virus suspension of a titer of $10^{-5.8}$, while another 8 mice were similarly injected in the peritoneal cavity. As controls for the tumor-bearing mice, 16 normal mice received 0.2 ml. of the same inoculum, 8 intraperitoneally and 8 subcutaneously. The group which received the intraperitoneal inoculation began to die from the fifth day of infection. On the sixth day, two moribund mice from each group were sacrificed and the LD_{50} of various tissues was measured. As shown in Table 2, the multiplication of the virus was demonstrable in the neoplasm when the virus was inoculated directly into the tumor or indirectly into the peritoneal cavity. At the same time the LD_{50} of spleen and liver seemed to be higher in the virus-infected tumor-bearing mice than in the virus-infected but otherwise normal animals.

In the next experiment the growth pattern of the virus was examined in the tumor and other tissues beginning one day after infection. A 0.1 cc. of a 10^{-2} suspension of the

virus (LD_{50} $10^{-4.5}$) was inoculated intratumorally into 10 mice bearing growths eight days old, while another 10 mice were inoculated with the same material in the peritoneal cavity. As controls, two groups of normal mice received either intraperitoneal or subcutaneous inoculations of the virus as in the previous experiment. On 2, 5, and 8 days after virus inoculation, two mice from each group were sacrificed and the LD_{50} of liver, brain, and tumor was measured. As shown in Table 3, the LD_{50} of the tumor was higher after direct intratumoral inoculation than after intraperitoneal inoculation, even though the LD_{50} of the liver was almost at the same level in both

TABLE 2. MULTIPLICATION OF ORNITHOSIS VIRUS IN TISSUES OF NORMAL AND CARCINOMA-BEARING MICE

	Virus titer in tumor-bearing mice			*Virus titer in normal mice*	
Dilution of tissue extract	*Liver*	*Spleen*	*Carcinoma*	*Liver*	*Spleen*
	Intratumoral inoculation			*Subcutaneous inoculation*	
10^{-1}				5, 5, 5, 6	5, 5, 5, 5
10^{-2}				6, 6, 6, 6	5, 6, 6, 6
10^{-3}	5, 6, 6, 7	6, 6, 6, 7	6, 6, 6, 7	Not tested	Not tested
10^{-4}	7, 7, 8, 9	6, 7, 7, 8	6, 8, 9, 10		
10^{-5}	9, 0, 0, 0	8, 9, 0, 0	0, 0, 0		
	Intraperitoneal inoculation			*Intraperitoneal inoculation*	
10^{-2}			6, 6, 7, 7		Not tested
10^{-3}	5, 5, 5, 6	5, 6, 6, 6	8, 10, 10, 0	5, 6, 7, 8	
10^{-4}	6, 7, 7, 7	6, 7, 7, 7	0, 0, 0, 0	7, 7, 7, 8	
10^{-5}	7, 7, 9, 9	8, 9, 10, 0		8, 0, 0, 0	

Numbers represent days of mouse death; 0 surviving mice.

cases. These facts may indicate that the infective titer of the tumor was not merely an expression of secondary growth of the virus which multiplied first in the liver, but rather an expression of propagation *in situ* of the virus following intratumoral inoculation. When the ornithosis virus was inoculated into the carcinoma, the infective titer of the tumor attained its maximum on the fifth day, and on the eighth day the LD_{50} of the tumor was still $10^{-4.0}$. On the other hand, the LD_{50} of the liver decreased from $10^{-5.0}$ to $10^{-2.8}$ on the eighth day. The dissociation of virus titer in the liver and the carcinoma is an interesting problem which will be discussed later.

2. *Experiments with sarcoma.* Using 0.1 ml. of 10^{-1} virus suspension (LD_{50} $10^{-4.2}$) intratumoral inoculations were made in 10 mice bearing growths 6 days old, while another 10 mice were injected with the same material in the peritoneal cavity. On 2, 5, and 10 days thereafter, two mice from both groups were sacrificed and the LD_{50} of liver and sarcoma was measured. As indicated in Table 4, the ornithosis virus showed almost the same degree of affinity for the sarcoma as for the carcinoma. When the virus was inoculated intraperitoneally, the LD_{50} of the liver was $10^{-4.8}$ on the 5th day

of infection and on the 10th day it decreased to $10^{-3.0}$. On the contrary, the LD_{50} of the sarcoma was $10^{-4.0}$ on the 5th day of infection and by the 10th day it increased to $10^{-5.0}$. In this experiment, therefore, the dissociation of infective titer in the liver and the tumor was as evident as in the experiment with the carcinoma.

TABLE 3. MULTIPLICATION PATTERNS OF ORNITHOSIS VIRUS IN TISSUES OF NORMAL AND CARCINOMA-BEARING MICE

	Virus titer in tumor-bearing mice				*Virus titer in normal mice*	
Days after inoculation	*Liver*	*Brain*	*Carci-noma*	*Route of inoculation*	*Liver*	*Brain*
2	1.0	<1.0	2.3	intratumorally or subcutaneously	<1.0	<1.0
	3.3	<1.0	1.0	intraperitoneally	2.8	<1.0
5	5.0	1.8	3.8	i.t. or s.c.	<2.0	<1.0
	5.3	1.3	3.0	i.p.	>4.5	1.3
8	2.8	<1.0	4.0	i.t. or s.c.	<2.0	<1.0
	3.5	<1.0	2.8	i.p.	4.5	<1.0

Figures are log of LD_{50}.

TABLE 4. MULTIPLICATION OF ORNITHOSIS VIRUS IN LIVER AND TUMOR OF SARCOMA-BEARING MICE

		Sarcoma-implanted mice	
Days after inoculation	*Route of inoculation*	*Liver*	*Tumor*
2	intratumorally	2.3	3.3
	intraperitoneally	4.0	2.0
5	i.t.	4.8	4.0
	i.p.	5.0	3.0
10	i.t.	3.0	5.0
	i.p.	3.0	2.0

Figures are log of LD_{50}.

INFLUENCE OF VIRUS INFECTION ON THE RATE OF TUMOR GROWTH

In the experiments described above, there were almost no differences in the growth rate between the non-infected and virus-infected tumors, but the influence of the presence of virus on tumor development was investigated further.

1. *Carcinoma.* An intratumoral inoculation of 0.1 cc. of a 10^{-1} suspension of virus was given to 10 mice bearing nine-day tumors. Another 10 normal mice bearing tumors of the same age served as controls (tumor generation I of Fig. 1). Four days

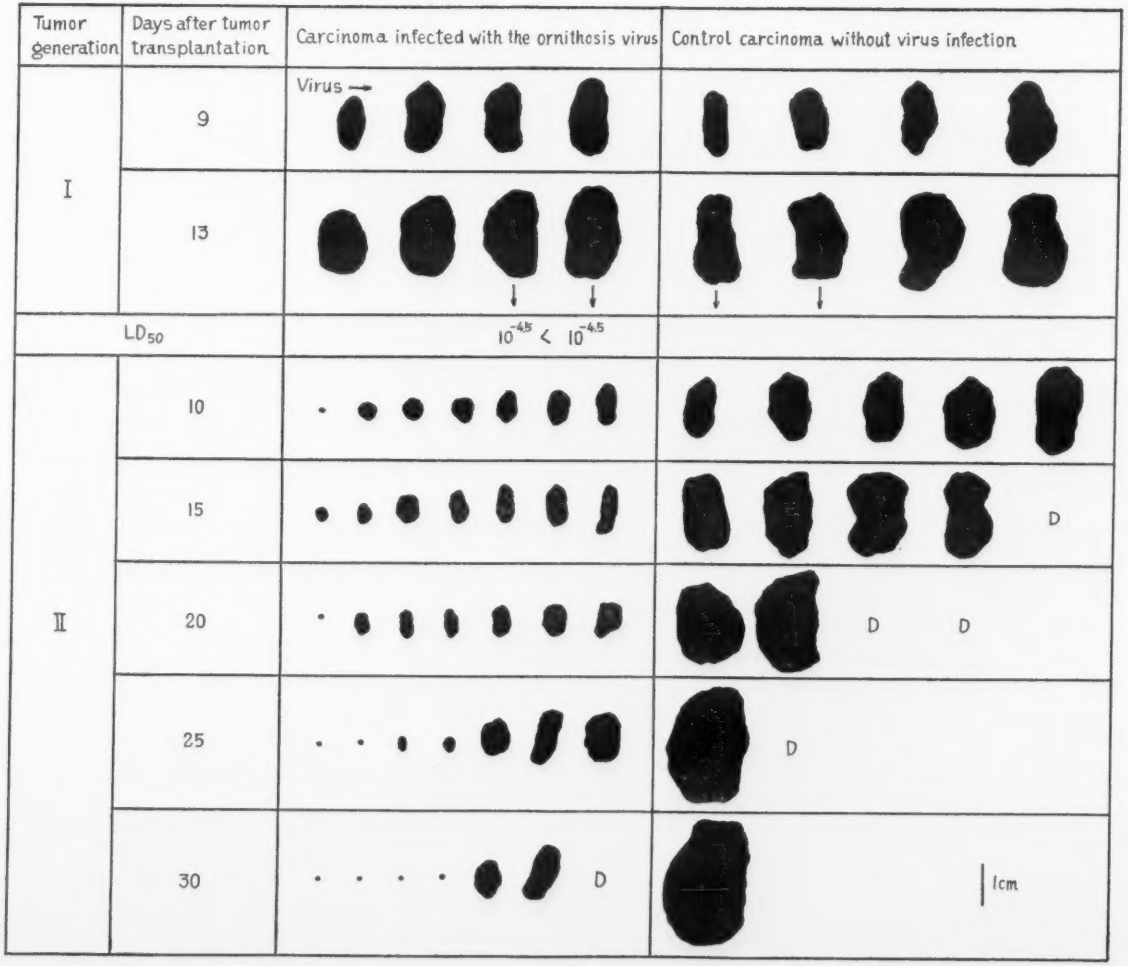

FIG. 1. Influence of ornithosis virus infection on the development of quinone-induced carcinoma.

after virus infection two mice from each group were sacrificed and their tumors, which had a virus titer of $10^{-4.5}$, were transplanted into another group of seven mice (tumor generation II of Fig. 1). As shown by the average size as depicted in Fig. 1, the development of the virus-infected carcinoma was not affected in the first generation, but growth was markedly inhibited in the second generation as compared to the non-infected tumor. Further, some of the virus-infected tumors underwent regression. This experiment was repeated under exactly the same conditions, and the same results were obtained.

2. *Sarcoma.* The experiments described for carcinoma were repeated with a sarcoma. The age of the tumor, mode of infection with the virus, passage to a second generation, and total number of animals inoculated were as described for the preceding carcinoma studies. The results, too, were quite comparable for there was little effect of the tumors directly infected with the virus. However, in the second generation, the sarcoma showed more resistance to virus infection than did the carcinoma, whereas in the third generation there was a pronounced inhibition of growth in the tumors themselves throughout the 35-day period of observation (Fig. 2).

INFLUENCE OF HOST IMMUNITY UPON THE PROPAGATION OF THE ORNITHOSIS VIRUS IN THE TUMOR

A total of 30 mice were immunized by the intraperitoneal inoculation of 0.2 ml. of 10^{-2} virus suspension and 15 days later were grafted with the carcinoma. Normal mice were also grafted with the tumor as controls. On the eighth day after tumor transplantation, 15 tumor-bearing mice of the immunized group received intratumoral injections of 0.1 cc. of a 10^{-1} virus suspension, while the other 15 mice received similar inoculations intraperitoneally. On 2, 5, 8, and 14 days after virus inoculation, the size of the tumors was measured, two mice from each group were sacrificed, and the LD_{50} of both livers and tumors was estimated. The results are presented in Table 5. Contrary to what might be anticipated, there was no virus multiplication in the immune host even if the injection had been intratumoral.

The possibility that tumor cells themselves are not susceptible to the virus became an important consideration. Accordingly, carcinomas, which had developed in the virus-immune mice, were transplanted to normal mice. Eight days later 0.1 ml. of a 10^{-1} virus suspension was inoculated into tumors, and on the eighth day after the virus infection the virus titer of the two tumors was investigated. The LD_{50} of the tumor was found to range from $10^{-4.0}$ to $10^{-4.5}$, that is, titers almost equal to those of normal tumors. From this experiment we may conclude that the virus susceptibility of the tumor cells themselves does not change following transplantation of the tumor into normal mice.

VIRUS NEUTRALIZING POWER OF SERUM AND TISSUE EXTRACTS FROM MICE IMMUNIZED TO THE VIRUS

This study was carried out in a further attempt to ascertain why the virus failed to grow in the tumors of immune mice. A group of 30 mice was immunized by two intraperitoneal inoculations of 0.2 cc. of a 10^{-2} suspension at a weekly interval, and two weeks after the second inoculation they were grafted with the carcinoma. After another two weeks, when the tumor was well developed, blood was collected from each mouse

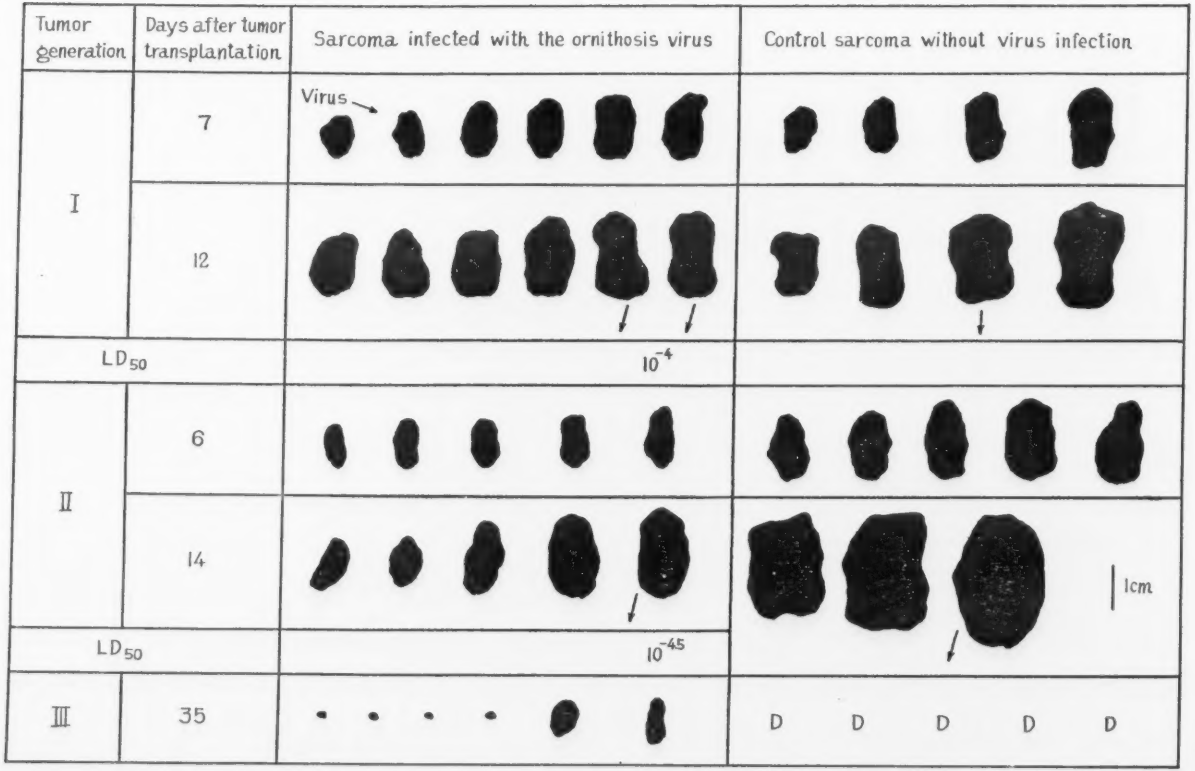

FIG. 2. Influence of ornithosis virus infection on the development of fructose-induced sarcoma.

by cardiac puncture and the samples pooled. At the same time the tumors and the livers were removed from five mice and 20 per cent extracts were obtained. The suspensions were centrifuged at 3,000 rpm. for 20 minutes, the supernatants removed and then refrigerated for 10 days before being used in titrations of virus neutralization. As

TABLE 5. INFLUENCE OF HOST IMMUNITY ON THE PROPAGATION OF ORNITHOSIS VIRUS IN TUMOR AND LIVER

Days after inoculation	*Route of inoculation*	*Normal mice*		*Immunized mice*	
		Liver	*Carcinoma*	*Liver*	*Carcinoma*
2	intratumorally	2.0	2.5	1.5	<1.0
	intraperitoneally	2.0	<1.0	1.8	<1.0
5	i.t.	5.0	4.8	3.0	1.8
	i.p.	3.8	2.8	<1.0	<1.0
8	i.t.	3.5	5.0	<1.0	<1.0
	i.p.	3.0	2.0	<1.0	<1.0
14	i.t.	2.0	3.8	<1.0	1.5
	i.p.	1.5	1.5	<1.0	<1.0

Figures are log of LD_{50}.

TABLE 6. NEUTRALIZING ACTIVITIES OF TISSUE EXTRACTS AND SERUM FROM MICE IMMUNIZED AGAINST ORNITHOSIS VIRUS

Material tested		*Virus dilution*				
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	LD_{50}
Serum	Control mice	+5/5	5/5	5/5	2/5	$10^{-5.8}$
	Immunized mice	5/5	5/5	3/5	0/5	$10^{-5.3}$
Liver extracts	Control mice	5/5	5/5	5/5	1/5	$10^{-5.7}$
	Immunized mice	5/5	5/5	2/5	0/4	$10^{-4.8}$
Tumor extracts	Control mice	5/5	5/5	5/5	1/5	$10^{-5.7}$
	Immunized mice	5/5	5/5	5/5	1/5	$10^{-5.7}$

+ Mice died. / Mice inoculated.

shown in Table 6, serum and liver extracts of immunized mice exhibited slight virus-neutralizing power, but no such activities were detectable in the carcinoma extracts. These results were confirmed by other similar experiments. From these results we may postulate that the failure of virus to grow in the carcinoma is not due to a neutralizing substance contained in the tumor.

TRANSPLANTATION OF VIRUS-CARRYING TUMOR TO THE IMMUNE MICE

Contrary to the tumor-producing viruses, extraneous viruses often disappear from the tumor when the latter is transplanted to an immune host.[8] Since it is logical to think of the serum antibody as a factor here, and aware that the ornithosis virus elicits

only low titer of neutralizing antibody in mice, we decided to investigate whether the ornithosis virus in our mouse tumors behaved like other passenger virus and tumor systems.

Accordingly, mice were immunized with 0.2 ml. 10^{-3} virus suspension and 27 days later the carcinoma, which had been virus infected four days previously, was transplanted into six immunized and six normal mice. The immunity of these mice to the ornithosis virus was checked by the intracerebral challenge test. Eight days after tumor transplantation, two mice were sacrificed from both groups and infectivity of extracts of the pooled tumors and livers was measured: the LD_{50} of the tumor grown in the immune host was 10^{-1}, whereas that of the tumor grown in normal mice was $10^{-4.5}$. Thus, the growth of the ornithosis virus in the tumor cells was markedly suppressed by transplanting the tumor into mice immune to the virus, although virus-infected carcinomas developed almost as well as non-infected tumors in these mice.

DISCUSSION

The lymphogranuloma venereum virus was first propagated in the Ehrlich tumor by Schoen[17] and was maintained for several generations. The present experiments show that an allied virus, that of ornithosis, propagates well in both a carcinoma and a sarcoma of the mouse. However, these tumors when infected with the virus could not be transplanted serially over three generations. It would seem that one cannot generalize on interrelations of viruses and tumors, however, for although the virus is not infective for the rat by intraperitoneal route, it can be passed serially through the rat ascites tumor for ten generations.[8] On the other hand, the Col-SK and western equine encephalomyelitis viruses decrease considerably in titer or may completely disappear in about 10 days as found in our studies with two mouse tumors.[9]

Following intraperitoneal or intratumoral inoculation of the virus, a marked dissociation was observed in the infectivity of liver and tumor. In the liver there was a marked decline in the virus titer, a fact no doubt due to the onset of the immunological response, while the infectivity in the tumor decreased very slowly. Could one explain the phenomenon, as suggested in another virus-tumor sequence, by a lack of immunological response of the tumor cell? Perhaps, but the long persistence of the virus in the tumor may be due to continuous cell proliferation which creates optimal conditions for virus multiplication.

As in Levaditi's studies with rat tumor, the ornithosis virus could not be propagated in tumors grafted in immunized mice. In this respect the virus behaved as a typical extraneous or passenger virus such as has been described by several authors.[1, 20] The virus multiplication is evidently an intracellular event, but there are fundamental differences between tumor-causing and passenger viruses especially in the degree of association with

the tumor cells. Immune hosts do not lose their inherent power to support virus growth, since the virus multiplies in "immune tumors" transplanted to normal hosts as well as in ordinary tumors. It is clear, therefore, that the tumor passively shares the immunological properties of the host's tissues.

The fact that the ornithosis virus thrived in the tumor cells after the appearance of immunity in the host but failed to grow in the tumor grafted into immunized mice, may be interpreted as follows: if the neutralizing antibodies in the serum of mice immunized with the ornithosis virus is low, the tumor, nevertheless, is continuously rinsed by the blood stream and minute amounts of antibodies may be present to prevent the virus from attaching and penetrating the tumor cells. In other words, the general postulate of virology that an immune serum is not effective after the infection has been established, although it may be effective before, may well be applied here.

SUMMARY

1. Ornithosis virus multiplied abundantly in two transplantable mouse tumors following intraperitoneal or intratumoral inoculation, the virus titer remaining quite high in the tumor at a time when it was declining in the liver.

2. Transplantability of the virus-infected tumors was far below that of non-infected tumors, serial transplantation being unsuccessful beyond three passages.

3. Ornithosis virus failed to propagate in tumors growing in hosts immunized to the virus.

4. A virus-neutralizing power, though weak, was demonstrated in serum and in liver extracts of immunized mice, but no such power was observed in extracts of tumors growing in the same hosts.

5. When the virus-infected tumor was transplanted into immunized mice the multiplication of the virus was suppressed and the tumor grew relatively well. However, when the same tumor was transplanted into normal mice, the virus multiplied and tumor growth was remarkably suppressed.

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A RIGID MEMBRANE FOR MEASUREMENT OF COLLOIDAL OSMOTIC PRESSURE WITH THE HEPP OSMOMETER**

INTRODUCTION

Perhaps the simplest way to measure the colloidal osmotic pressure of a protein solution is to put that solution inside of a collodion bag, to dip the bag into the solvent, and then wait until the hydrostatic pressure developed inside of the bag, measured by the height of a column of solution, becomes constant and equal to the osmotic pressure of the proteins. This method has two main disadvantages: (i) it takes several days before an equilibrium is established so that the procedure must be carried out at low temperature in order to avoid any decomposition of the proteins; (ii) the final osmotic pressure is not that of the original sample but that of a solution diluted by the water that has passed across the membrane. Since the relationship between protein concentration and osmotic pressure is not a linear one, a simple proportion does not give the correct value for the osmotic pressure of the original sample.

To avoid both these disadvantages, different types of osmometers have been devised in which the osmotic pressure is equalized by a positive pressure applied on the protein solution or by a negative pressure applied on the solvent; the pressure that stops the passage of water across the membrane is equal to the osmotic pressure of the protein solution. As an ulterior development of this technique, Hepp[2] devised an osmometer in which transfer of water through the membrane is measured in terms of the motion of a meniscus between air and ultrafiltrate. The velocity is plotted against the applied difference of pressure and the value of the osmotic pressure is obtained by interpolation, being equal to the difference of pressure at which the velocity of transfer is zero. With this type of osmometer, once the diffusible ions have reached an equilibrium, it takes just a few minutes to measure the osmotic pressure. The accuracy of the method depends upon the accuracy with which the velocity of water transfer through the membrane can be measured. The volume of water crossing

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the membrane per unit of time (V) is related to the applied pressure by the equation:

$$V = K\,S\,(P - P_o) \qquad (1)$$

where K is a constant of proportionality whose value is a measure of the permeability of the membrane, S is the surface of the membrane, P the applied pressure, and P_o the osmotic pressure of the protein solution. What is actually measured is not a displacement of volume but the linear velocity of the water (h) in a capillary of radius r. Since:

$$h = V/\pi r^2 \qquad (2)$$

we have:

$$h = K\,S\,(P - P_o)/\pi r^2 \qquad (3)$$

To increase the accuracy, h must be increased, and this can be done by increasing K and S and decreasing the radius of the capillary. However, K cannot be increased beyond a certain limit without making the membrane permeable to proteins. The value of K is such that in order to have a velocity of the order of 0.1 mm. per minute for $P - P_o = 10$ mm. H_2O, i.e., a velocity measurable with sufficient accuracy under the microscope, the ratio $S/\pi r^2$ must be about 1500. Under these conditions it is of primary importance to have the membrane as rigid as possible. If the membrane is not rigid, it moves when a difference of pressure is applied, and this movement displaces water in the capillary. Actually, if an unsupported membrane is used, the displacement of water due to the movement of the membrane may be greater than that due to the transfer of water through the membrane, so that the measurement of this last quantity is of doubtful accuracy. This difficulty might be avoided by casting the membranes on a rolled nickel screen, as suggested by Fuoss and Mead.[1] In the present work sufficient rigidity for accurate measurements was achieved by the use of membranes prepared on a flat sheet of perforated stainless steel.

THE APPARATUS

The general scheme of the apparatus is that described by Hepp[2] and more recently by Rehm,[3] with some simplifications in the details. A schematic cross section of the apparatus is given in Figure 1. The solvent fills the capillary C and the pores of the filter paper F beneath the membrane. The membrane M is placed on the filter paper and pressed against the Lucite plate A by means of ring B, made of Lucite also. Eight nuts screwed on bolts fixed to the Lucite plate hold firmly B against A. Two rubber gaskets, R, above and below the membrane, make the system watertight. The protein solution to be analyzed is placed inside of the ring B on the membrane M. A watch-glass, G, is placed over the ring to prevent evaporation of the solution. The velocity of water transfer through the membrane is observed with a microscope equipped with an ocular micrometer graduated in tenths of mm. The magnification of the microscope

used is about 300 diameters. The capillary is connected to a bottle, two liters or more in capacity, in which the air pressure can be adjusted. In order to have a constant pressure, one must have air at a constant temperature in the bottle and avoid irradiation from sources of heat (for instance, the light of the microscope). The pressure is read on a water manometer connected with the bottle. The apparatus is held in the proper position by a common laboratory clamp, H, and in addition the capillary is fixed to the stage of the microscope by two clips.

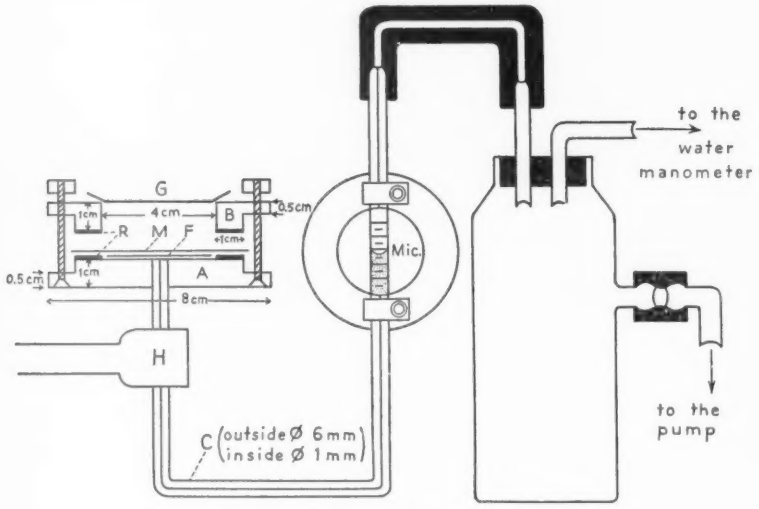

FIG. 1. Diagram of the apparatus described in the text.

THE MEMBRANE

The stainless steel support on which the membrane is laid down is a circular plate, 6.5 cm. in diameter and 0.5 mm. thick. Its holes have a diameter of 1.5 to 2 mm. and are placed 0.5 to 1 mm. apart from each other. The solution of collodion is prepared in the following way, as suggested by Scatchard *et al.*[4]: eight grams of nitrocellulose and 32 ml. of anhydrous ethylene glycol are placed in a dry bottle and shaken to coat the nitrocellulose with glycol. A mixture of 50 ml. anhydrous ether and 150 ml. absolute alcohol is added. The mixture is shaken, allowed to stand overnight, and then thoroughly mixed.

A Petri dish, 14 cm. in diameter, is placed inside of a box or desiccator through which flows a current of dry air. The dish is filled with mercury, and a glass plate, 10 cm. in diameter, is floated on the mercury. In the

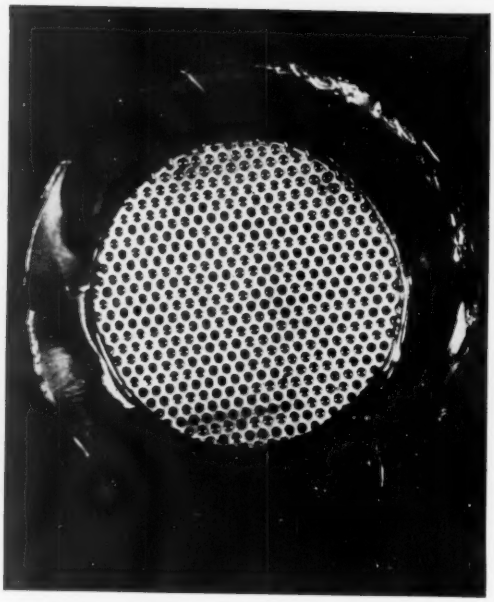

FIG. 2. Photograph of a collodion membrane made on a perforated stainless steel plate according to the procedure described in the text.

middle of the glass plate is placed the steel plate. Ten ml. of the collodion solution are poured on the middle of the steel plate with a pipette from which the tip has been cut off. In about five minutes the solution distributes itself evenly on the surface of the glass plate. The steel plate is completely submerged and the collodion solution has replaced the air in the holes of the steel plate. The membrane is left in the box to dry for 48 hours. After that, the glass plate is immersed in tap water. In about 10 minutes the collodion membrane attached to the steel plate loosens from the glass plate. After the membrane has been washed for five hours or more in running water to remove the glycol, it is ready to be used. The surface that was against the glass plate is smooth; the surface which was exposed to the air presents a depression corresponding to each hole of the mesh. The membrane is stored under water and it is never allowed to dry. Figure 2 is a photograph of a membrane on its plate; only the central part of the supported area is exposed to solution in the osmometer.

ASSEMBLING OF THE APPARATUS

To avoid formation of air bubbles in the filter paper or in the capillary when a negative pressure is applied on the solvent, the solvent must be air-free. About 50 ml. of the solvent (0.9% NaCl solution in case of serum or plasma) and two circular pieces of filter paper (Whatman No. 41), 3.9 cm. in diameter, are placed in a flask and extracted by vacuum for about five minutes. The capillary, thoroughly cleaned, is filled with the air-free solution; its tip is greased externally and inserted in A so that the capillary is flush with the surface of the plate.

Next, a rubber gasket is placed on the plate and made adherent to it by means of a thin film of grease. About one ml. of the solvent is then placed on the plate and the two air-free pieces of filter paper are superimposed and placed on the plate, care being taken that no air bubbles are trapped in between. More air-free solution is added to the surface of the filter paper and then the smooth face of the membrane, previously washed with saline solution, is placed on the filter paper. A rubber gasket is placed on the membrane and ring B is pressed against plate A by means of the eight nuts. The nuts are screwed as tight as possible by hand and a final turn is given with pliers. The membrane is covered with air-free solution and the capillary connected to a vacuum pump (about 200 mm. Hg depression) for about 10 minutes. During this time no air bubble must appear in the capillary, for if any air bubble is seen in the capillary, the apparatus has to be assembled again.

DETERMINATION OF THE BLANK

Two ml. of the solvent (isotonic solution in case of serum or plasma) are placed over the membrane and the watch-glass is placed on the ring. By applying a positive or a negative pressure, depending upon the actual position of the meniscus in the capillary, the meniscus is brought into the field of the microscope. The pressure is then adjusted so that the meniscus does not move or moves very slowly. After 30 minutes the pressure is changed by about 20 to 40 mm. H_2O and the time required for the meniscus to travel between two chosen marks of the graduated scale is recorded. The pressure is then changed so that the meniscus travels in the opposite direction and the velocity of the meniscus is measured again. Despite the rigidity of the membrane, it is found that a sudden change of the applied pressure produces some microscopic movement of the meniscus that has nothing to do with the transfer of water through the membrane. For this reason the movement during the first 30 seconds is not recorded. The two marks on the graduated scale are chosen so that the time required for the meniscus to travel from one mark to the other is 150 to 250 seconds under a pressure difference of 20 mm. H_2O. Thirty minutes after the first two velocities are recorded, two other velocities are measured at the same or different pressures. The four points must give the same pressure value (within $\pm$ 0.5 mm. H_2O) for zero velocity; otherwise two further measurements must be made 30 minutes later. The 30-minute interval between one set of measurements and the other is sufficient to reveal whether or not the system has reached an equilibrium. The "blank" pressure is the algebraic summation of the hydrostatic pressure, due to a difference of level between the meniscus in the capillary and the water on the membrane, and the pressure due to capillarity. Once determined, the blank remains constant as long as the position of the capillary under the microscope is not changed and need not be checked before each measurement.

MEASUREMENT OF THE OSMOTIC PRESSURE

The solvent on the membrane is pipetted off and the upper surface of the membrane is blotted with filter paper: when only a film of moisture remains on the membrane, the volume of the outside solution starts to decrease by evaporation, as is indicated by the fact that the meniscus now travels toward the membrane. Two ml. of protein solution are placed on the membrane and the watch glass is replaced. If the solution is normal plasma or serum, the meniscus moves out of the microscopic field in about 20 seconds. As soon as possible a negative pressure is applied to bring the meniscus back to the

middle of the microscopic field. The pressure is then adjusted until the meniscus does not move or moves very slowly. This last operation is accomplished in two or three minutes. After that it is necessary to wait for the complete equilibration of the system. After 90 minutes two readings of velocity are taken and two others 30 minutes later, as described for the blank. The four velocities are plotted against the applied pressures and the osmotic pressure is obtained by interpolation. An example of two measurements carried out on the same plasma but with different membranes and osmometers is given in Figure 3; pressures of —318 and —320 mm. H_2O were obtained for velocities equal to zero. The value of the blank was +29 mm. H_2O for the first osmometer and +28 for the second, so that the values for the osmotic pressure are 347 and 348 mm. H_2O respectively. In my experience the Hepp osmometer equipped with an unsupported collodion membrane gives results subject to an error about five times as great as that of data obtained with the rigid membrane.

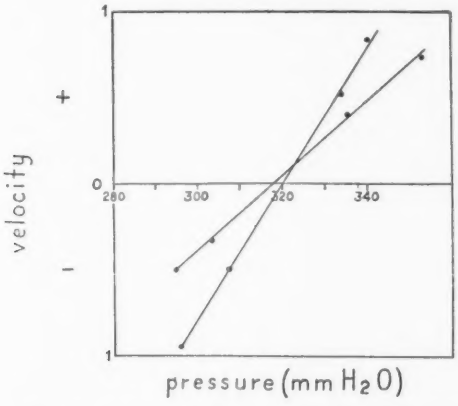

FIG. 3. Velocity of the meniscus (in arbitrary units) plotted against the pressure difference on the two sides of the collodion membrane.

When the measurement is finished, the protein solution is pipetted out and replaced by 10 ml. of saline solution. After an hour this is replaced by fresh solution and the washing is repeated at least three times. After the washings, the blank has returned to its original value and a new measurement may be undertaken. The same membrane can be used for many measurements. For example, one membrane, with which 10 measurements have been made over a period of about two months, is still satisfactory. When the apparatus is not in use the membrane must always be covered by water; a crystal of thymol added to the water prevents bacterial contamination. When a new membrane has to be made, the steel plate is easily recovered by allowing the membrane to dry: the collodion membrane then detaches itself from the plate.

MICROANALYSIS

When less than 2 ml. of protein solution is available, the measurement of the colloidal osmotic pressure can still be performed by reducing the area of the membrane and the diameter of the capillary. If the measurement has to be carried out on only 0.2 ml., the inside diameter of ring B is reduced to 15 mm. and the inside diameter of the capillary to 0.25 mm. Since the ratio of the membrane area to the cross section of the capillary is more than doubled, the velocity of the meniscus in the capillary for a given difference of pressure is higher than in the apparatus for 2 ml. samples.

SUMMARY

The accuracy of the Hepp osmometer has been improved by the use of a collodion membrane made on a perforated stainless steel plate. The procedure for measuring the colloidal osmotic pressure of a protein solution with the new osmometer is described.

ACKNOWLEDGMENT

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BOOK REVIEWS

CORONARY HEART DISEASE IN YOUNG ADULTS—A MULTIDISCIPLINARY STUDY. By Menard M. Gertler and Paul D. White, with the aid, advice, and editorial assistance of E. F. Bland, J. Fertig, S. M. Garn, J. Lerman, S. A. Levine, H. B. Sprague, and N. C. Turner. Cambridge, Mass., Harvard University Press, 1954. xviii, 218 pp. $5.00.

As coronary heart disease increases in incidence, the need for a better understanding of the factors involved in the origin of atherosclerosis, and especially of coronary artery disease, becomes more and more compelling. Statistical studies of human material and experimental studies on animals have furnished certain clues concerning coronary heart disease which required more intensive study in order to "unite in a more cohesive fashion the various disciplines from which these clues stemmed." The previous experimental and clinical observations gave rise to the following viewpoints which therefore directed this study: the relation of sex, the morphological, genetic, athletic, occupational, physiological, psychological, clinical, dietary, hormonal, and biochemical aspects. One hundred patients under 40 with coronary heart disease were studied intensively and compared with unmatched as well as matched pair controls of 97 males.

Though the book is small, its scope is very broad as can be seen from the many authors contributing to its final form. It contains 61 tables, 26 figures. There are six appendices containing all the data from which the tables were made and the conclusions drawn. In it we find the Terman-Miles masculinity-femininity test, the hereditary records, summary of hereditary data, diet records, summary of case histories, and finally an appendix on follow-up study of coronary and unmatched control groups.

There are eleven chapters in all, some of which cover clinical appraisal of the coronary group, the rôle of heredity (and here strangely enough the word "race" is used interchangeably with "national origin"). There are chapters on anthropometric and morphological appraisal of physique, athletic activity, and occupation, findings on masculinity, endocrine and biochemical factors, and finally there is a chapter on oxidation-reduction potentials of saliva of coronary and normal patients.

Some of the results fully substantiate clues already in existence. The preponderance of males, 97 out of 100, the identity of the clinical picture of the disease in young and old, the rôle of heredity in the etiology of coronary artery disease are well documented although the mode of inheritance is not clear. The authors describe a body type more prone to coronary heart disease, the so-called endomorph-mesomorph, a heavily built, bony, muscular, and sturdy male. This was foreshadowed in a paper in 1937 by Glenby, White and Levine.

There emerges the existence of a metabolic derangement in these patients but so complicated that all the threads are not yet unravelled. No support is here found for the fear of cholesterol ingestion, since the coronary heart disease group showed a higher serum cholesterol although they ingested less than the control group. The higher serum cholesterol levels were associated with altered serum lipid fraction relationships. The authors believe that the endogenous cholesterol plays a definite rôle in the cause of coronary

artery sclerosis. Many of the patients also showed an elevated blood uric level, thus further emphasizing the variety of metabolic derangements involved in this important disease.

The rôle of habits and certain life situations in the genesis of coronary heart disease could not be ascertained from the authors' data. The coronary heart disease group did consume more tobacco than the control group, however, and more recent statistical studies on a very large scale do implicate smoking in the etiology of this disease. Among the unsolved problems there remain the rôle of the thyroid and adrenal glands in the genesis of coronary heart disease.

That these studies are necessarily an elaboration of already existing ideas on the genesis of coronary heart disease is merely a recognition of the limitations imposed on any study by the method of statistical analysis. This book is a model of the permissibility and limitations of statistical approach in the study of clinical problems. Even though new clues have not been uncovered, the old ones have certainly received extensive study. But the main purpose of the book, correct preselection of coronary-prone patients, and a definite form of replacement or adjuvant therapy that could be effective in delaying or preventing the onset of the disease, remains unrealized. The authors appreciate that adequate understanding of the nature and causes of coronary heart disease is the key to their problem and must precede the question of preselection and therapy. Perhaps the solution of the problem of coronary heart disease will be found, not by a massive statistical assault, but though the still, small voice out of the experimental laboratory.

LOUIS NAHUM

EMOTIONS AND BODILY CHANGES. By Flanders Dunbar. New York, Columbia University Press, 1954. 4th ed. xxii, 1192 pp. $15.00.

The appearance of the original edition of this work in 1935 is historically significant. Dr. Dunbar is a psychoanalyst who has a broad interest in medicine, physiology, and research method. Her pioneer spirit is recognized in all of her writings as well as in her career: she was the principal founder of the journal, *Psychosomatic Medicine,* and indeed must be given credit for coining the term, "psychosomatic." As originally conceived, this book was intended as a bibliography of material pertinent to the study and understanding of the relationships between psychological diculties, physiological function, and pathological process. The first such compendium, it was badly needed and widely welcomed. But it was and is more than just a bibliography. Between the customary introductory and therapeutic considerations and conclusions sections is the main body of the work, divided into chapters by systems. Although loosely organized and mainly clinical, illustrated largely by cases and the experiences of many authors, it is profoundly provocative. Readers are told just enough of an author's work to stimulate interest in looking up the work itself. Criticism is at a minimum and occurs chiefly in the selection of papers for discussion, and the length of discussion given, rather than in appraisal of methodology or results.

The impact of this approach is less powerful today than it was in 1935. The presentation of author after author, symptom after symptom, system after system builds to the acceptance of a "psychosomatic concept," the overthrowal of barriers between tight compartments labelled "mind," "body," "normality," and "illness." The student today comes to literature believing he already has such a concept. Such has been the effect of the teaching of Dunbar and those who followed her. He may wish to know (as many of my students do) what is the theory? What are the dynamics? Where is the evidence, the systematic research? What is the treatment? Which patients may be treated by a general practitioner, or a non-psychiatrist specialist?

Unfortunately, the new edition does not go far in answering these questions. It has grown mammoth in size (there are over 5,000 references) but has not changed in design. In fact, most chapters now have two subdivisions: the first is apparently the text of the 1938 edition covering the literature from 1910-1935, and the second covers the material from 1935-1953. Most of the important clinical studies are mentioned. There is some emphasis on personality profiles, a preferential point of view of the author's, but little reference to the current controversy over "specificity," "profiles," and "constellations" is made. It remains a valuable reference book, but has lost some readability with its increase in size. The section on treatment has been rewritten and serves as an introduction to the problem of obtaining help for patients rather than giving treatment methods in detail.

Perhaps it is to Dr. Dunbar's credit that her teaching, research, and stimulation of others has helped create an area of interest and work, now so large that it can no longer be adequately reviewed in one volume.

MARTIN L. PILOT

THE PSYCHOLOGY OF PERSONALITY. By Bernard Notcutt. New York, Philosophical Library, 1953. 255 pp. $4.75.

The author of this book states that his purpose "is more to evaluate than to describe the activities of psychologists." With this objective in mind Dr. Notcutt divides the major theories into three groups; those which concern themselves with basic traits, those which are primarily environmentalistic in orientation, and those which deal with the interaction between people and the environment. The techniques which have been developed in conjunction with these approaches are considered in the ensuing chapters. The final chapter, a brief sixteen pages, is devoted to a discussion of the development of the concept of personality since the time of the ancient Greeks.

One cannot help wondering to whom this little book is addressed. It is too superficial, too cursory, for specialists and yet its failure to provide any real summary makes it of relatively small value to the student or interested layman. In short, it appears that the book neither evaluates nor describes. It may also be noted that although they may make the book more readable, flippant remarks such as "it is possible to think of experimental psycho-

dynamics as being mainly concerned with providing quotable examples for use in lecture," or "psychoanalysts, making it their mission to shock people . . ." do not enhance the work.

P. B., JR.

BIOCHEMICAL DETERMINANTS OF MICROBIAL DISEASE. By Rene J. Dubos. Cambridge, Mass., Harvard University Press, 1954. 152 pp. $3.50.

This is a very stimulating book, since in it the author discusses infectious processes in biochemical terms. He emphasizes not only what can be done to develop our understanding of microbial disease and host resistance mechanisms, but also shows how meagre our knowledge in this field really is. Early in the book Dubos points out that current infectious disease literature has "a quaint mid-nineteenth century flavor. It describes disease as a conflict between two opponents, one endowed with 'aggressive' weapons, and guilty of 'invasion,' the other 'mobilizing' various defense weapons. . . ."

After pointing out our ignorance of host-parasite relationships, Dubos demonstrates how inadequate our thinking is when we use the archaic terms of warfare. He does this by describing some of the advances the biochemical approach has produced in our knowledge of infectious diseases. Among other things, he discusses the metabolic changes produced in tissues by inflammation, and the effects of these changes on bacteria growing in the host. He also explains some of the reasons for decreased resistance in metabolic diseases such as diabetes.

In the third chapter, the author discusses biochemical disturbances produced in the host by infections. Here, for example, Pappenheimer's hypothesis that diphtheria toxin acts by inhibiting host synthesis of cytochrome b is taken up in some detail. The lecithinase action of *Cl. welchii* toxin is also discussed and the relation of this enzyme to the disease picture. The Schwartzman reaction is also partially explained from the metabolic point of view.

The last two chapters dealing with tuberculosis and immunity I did not find as stimulating as the rest of the book, although here again there is much useful information expressed in biochemical terms.

The book is not a complete review of our biochemical knowledge of infectious disease, nor does it pretend to be. For instance, Racker's work showing that mouse poliomyelitis virus inhibits glycolysis in brain is not mentioned, nor it there any discussion of the observation that diphtheria toxin lowers host reserves of energy-rich phosphorus compounds, presumably by inhibiting oxidative phosphorylation.

Dubos' book seems to me a very valuable one, since it cannot help but stimulate interest in the biochemical mechanisms of infectious diseases and host resistance. It should be of interest to clinicians, medical students, and of course to anyone primarily interested in medical microbiology, since it takes up in readily readable terms this aspect of infection so frequently ignored in textbooks of infectious disease.

GIFFORD B. PINCHOT

PHYSICAL AND CHEMICAL ASPECTS OF BASIC MECHANISMS IN RADIOBIOLOGY. John L. Magee, Martin D. Kamen, and Robert L. Platzman, Eds. Washington, National Research Council, 1953. viii, 145 pp. $1.00.

This little book is the transcribed account of a Highland Park conference on this subject. It was attended by a representative group of persons interested in these basic mechanisms and the discussions were free and informative. The material has been quite well edited and yet has kept the tone of free discussion.

This particular volume is somewhat physical and chemical in character and discusses rather closely the effect of ionizing radiation on water. The physical basis for the chemical results is fully discussed by many people, though no conclusions are reached. Many numbers which are not ordinarily found in the literature are given here, together with refreshing estimates of their probable correctness.

It is a book which can be read with interest by anyone who needs to know the fundamental sides of physical action in radiobiology.

E. C. POLLARD

EVOLUTION IN THE GENUS DROSOPHILA. By J. T. Patterson and W. S. Stone. New York, The Macmillan Co., 1952. 610 pp., illus. $8.50.

The truly remarkable extent of our knowledge of evolution among flies of the genus *Drosophila* is apparent in this volume by two of the biologists who have contributed so much to that knowledge. Twenty-five years ago it seemed most unlikely that *Drosophila,* whatever its value as a laboratory tool for genetics, would be of any great use in the study of evolution directly. Today the situation is very different as, indeed, the appearance of this work proclaims.

The information presented and conclusions reached are the consequences of the labors of many investigators: biologists of every persuasion, taxonomists, cytologists, geneticists, ecologists, biometricians, biochemists, physiologists, have contributed to the knowledge here summarized. While, as the authors emphasize, "no single genus can give us an adequate example of all the multitude of variables and their combinations found in the evolution of living forms," the genus *Drosophila* provides exceptionally favorable material for the study of evolution since it consists of a large array of species occupying numerous habitats, has used "most of the major genetic systems to effect this diversification," and possesses the giant salivary gland chromosomes known thus far only in the *Diptera.* Studies of genic and chromosomal variation within and between species, combined with systematic and distributional data concerning the better known groups provide the meat of this book. Serious students of genetics, cytology, and evolution will find the book fascinating as well as an invaluable guide to the very extensive and rapidly growing literature on the subject. It will be somewhat more difficult for the non-specialist and how much of it he will wish to tackle will depend a good deal on the nature and extent of his biological background.

The chapters on chromosome evolution, genic variation and balance, isolating mechanisms including the "insemination reaction," and hybridization contain information of interest to biologists generally. The group of species which has been most thoroughly investigated, the *virilis* group, is dealt with in a separate chapter which can be read more or less independently of the remainder of the book. A final chapter of comparisons and conclusions is also of general interest, for the *Drosophila* findings are compared with those in other organisms.

D. F. POULSON

ADVENTURES IN PHYSIOLOGY. By Sir Henry Hallett Dale. London, Pergamon Press, 1953. xvi, 652 pp. £6.6.

Under this title Sir Henry Dale, a Nobel Laureate in 1936 and one of the most distinguished biologists of this or any age, has arranged for the publisher, at the urgent request of his friends, a selection of his writings. Reports of original research and lectures, they deal with the two lines of investigation to which he devoted primary attention over a period of about thirty years, i.e., the rôle of epinephrine and acetylcholine in the transmission at synaptic and neuroeffector junctions, and the local and systemic actions of histamine as it participates in the responses of the tissues of an organism to environmental conditions that threaten the integrity of its cells.

The thirty papers selected for reprinting have been arranged in chronological order. They need neither introduction nor recommendation; each one of them has an established place in the literature of science. The first in the sequence is his classic study which appeared in 1906, "On some physiological actions of ergot," in which Dale demonstrated that preparations of ergot work selectively upon the effectors of the sympathetic system, i.e., producing a paralysis of the motor elements whilst sparing the normal function of those whose actions are inhibited by epinephrine; this differential effect was later shown to be due to ergotoxine.

The background material essential to an appreciation of the proper position of the first paper in the sequence to follow, the "events behind the scenes" of the published work, is related in charming style in an Introduction especially prepared for the Adventures that serves to provide the historical thread binding the individual papers into a unit—a unit representative of an active life in research. Reading the Introduction and the comments that are appended to the majority of the papers—to illuminate the past and point to the future—one cannot fail to remark on the frequency with which Sir Henry ascribes this or that advance to accidental rather than planned circumstances. How, for illustration, the decision to join the staff of the Wellcome Laboratories in 1904, coupled with Wellcome's suggestion that a study of ergot should be made when an opportunity could be found, led to a setting in which epinephrine was administered to an animal that had already received successive doses of ergot and so in turn to the discovery of how ergot paralyzed the structures excited by adrenaline without altering the responses of those inhibited, as described in the first paper. And the second paper in the series "The Action of Extracts of the Pituitary

Body," though not too relevant to the main lines of the two principal research themes, is included because it arose from an accidental observation. Number 8, "The Anaphylactic Reaction of Plain Muscle in the Guinea-Pig," is described as having arisen from one of the most fortunate accidents, when "a lucky encounter" put Dale on the trail of acetylcholine in extracts of ergot.

These are but a few of the accidents. A measure of a man of science must be his gift for turning accidents into meaningful experience, the gift for the discovery of things not sought, serendipity.

Viewing his own work from the vantage point of history, Sir Henry develops, in his comments, another parallel thought—how so frequently throughout the course of his work he and others were near to the discovery of the rôle of epinephrine and acetylcholine before the possibility of a chemical aspect to junctional transmission was systematically investigated as such. A long period frequently follows the annunciation of an hypothesis based upon sound experimental evidence before it is put to further experimental test; R. T. Elliott, Dale's lifelong friend to whom the "Adventures" are dedicated, first suggested in 1904 while he was George Henry Lewes Student at Cambridge, and two years before Dale's first paper on ergot, that the liberation of adrenaline by the arrival of impulses at sympathetic nerve endings might transmit their excitatory or inhibitory reaction to effectors, muscle, or gland.

The suggestion was largely ignored, at least it had no effect upon trends in investigation, until after World War I and then the idea of chemical transmission at neural effector junctions developed through a recognition of the similarity between the action of the parasympathetic system and acetylcholine. The nicotine and muscarine-like effects of acetylcholine discovered in an investigation of ergot extracts for muscarine led to the conclusion "that there is some degree of biochemical similarity between the ganglion cells of the whole involuntary system and the terminations of voluntary nerve-fibers in striated muscle on the one hand, and the mechanism connected with the peripheral terminations of craniosacral involuntary nerves on the other (paper 11, "The action of certain esters and ethers of choline and their relation to muscarine," 1914).

The stage was set, but direct evidence of a convincing character for a chemical mediator was not forthcoming until 1921 when Lowei described the action of the "Vagustoff" on the frog's heart. But no one had as yet demonstrated the existence of acetylcholine in animal tissues; its discovery in the spleen of the horse during a survey of organ extracts for histamine, as described in the 22d paper "The presence of histamine and acetylcholine in the spleen of the ox and the horse" (1929), was the stimulus for the exploration of its functional significance, a search in which Dale was joined by such able colleagues as Gaddum, Feldberg, and Brown with results now so well known.

Elliott's suggestion, like that of Professor Z. M. Bacq in 1934 that Cannon's Sympathin E might be nor-epinephrine, received much less attention than it deserved. An idea or schema to be useful cannot be too far in advance of contemporary thought; moreover, it appears to be less likely to

receive attention when it creates a pattern than when it fills a conscious need, as did the suggestion that nerve fibers be classified not in terms of their anatomical distribution, but in relation to chemical transmission which Dale made to the Physiological Society in 1933 when he introduced the words "cholmergic" and "adrenergic"; words that have since become firmly established in usage. Quite appropriately this short note has been included in the collection.

The usefulness of this new nomenclature was made abundantly clear in the Linacre Lecture given in the following May (1934) in the Physiological Laboratory at Cambridge when Dale reviewed the evidence for chemical transmission at neuroeffector and synaptic junctions and classified them, so far as the evidence permitted at that time, as adrenergic and cholinergic. This lecture was the first of a series of review-lectures Dale gave on chemical transmission; the last, given at the Mount Sinai Hospital in New York in 1937 (The William Henry Welch Lecture), presents the results of the rapid advances made at Dale's laboratories at the National Institute for Medical Research in Hempstead in the intervening three years and records the position at the time when he ceased to take an active part in th development of the subject. These lectures were selected for inclusion in the Adventures along with two others, The Croonian of the Royal Society (1920) on the Biological Significance of Amaphylaxis and the Croonian of the Royal College of Physicians (1929) on some chemical factors in the control of the circulation.

The lecture titles give some evidence of Sir Henry's versatility and his breadth of interest, but reference to the bibliography of his works at the end of the volume indicates a still wider range of topics that engaged his attention—capillary circulation, acid-base balance in blood, insulin, as well as dorsal root fibers in the frog.

A well-chosen photograph of Sir Henry forms the frontispiece of this record of his researches, or Adventures, as he has preferred to call them. By any title they form an impressive, valuable record that is, in this book, made of easy access to contemporaries and those to follow.

DONALD H. BARRON

FIFTY YEARS OF MEDICINE. By Lord Horder. New York, Philosophical Library, 1954. 70 pp. $2.50.

This book represents an expanded version of the Harben Lectures delivered at the Royal Institute of Public Health and Hygiene, London, in December 1952. It also reveals far more of the author, an outstanding figure in the English medical world, than of the development of medicine over the last half-century. Despite this deviation from the spirit of the title, Lord Horder has given a clear though highly personalized résumé of many aspects of contemporary medicine.

In reviewing the scientific accomplishments of fifty years, Horder particularly notes improvement in the technique of diagnosis, and reveals himself as a pioneer in clinical pathology. He is careful to relate advances in surgery to discoveries in other fields, notably in anesthetics and bacteri-

ology. He also recognizes the rôle of chemotherapy, endocrinology, and improved instruments for observing the patient.

His survey of current conditions is more detailed. Lord Horder is not only a physician interested in life and death, but also an adherent of such related causes as birth control and cremation. His interest in eugenics and biological control of illness has led him to try to get legal sanction for ". . . voluntary sterilization of carriers of hereditable disease. . . ." He feels voluntary euthanasia is a matter for public rather than professional concern, though he expresses his own doubts as to its value. He advocates ". . . an extended pathology . . ." to include new points of view dealing with environmental and psychosomatic factors, in preference to establishing these as special branches of medicine. He criticizes the National Health Service in some detail, citing several familiar points: the institution of the program by immediate fiat, continuing budgetary deficits, and the neglect of the general practitioner in planning functioning units. On the other hand, Lord Horder concedes the need for centralized direction of coordinated health services, and argues ideally for a more gradual approach to government sponsorship. He feels that this might have been done through a progressive increase in subsidization of hospitals and medical schools and an expansion of prevailing health insurance.

Though hardly to be defined as an historical summary, this little volume is a fluent and entertaining expression of one man's career.

WILLIAM B. WALKER

AUTOTROPHIC MICRO-ORGANISMS. B. A. Fry and J. L. Peel, Eds. New York, Cambridge University Press, 1954. xi, 305 pp. $5.00.

This volume is a collection of papers read at the Fourth Symposium of the Society for General Microbiology which was held in London in April 1954. The subject under discussion are those micro-organisms which utilize carbon dioxide as their chief or only carbon source. These organisms derive their energy either from the absorption of light or from the oxidation of inorganic compounds such as hydrogen, ammonia, sulfur, and ferrous salts. Van Niel has shown that a strict definition of "autotrophic" and "heterotrophic" is impossible, we must be content to consider organisms which primarily fix carbon dioxide or which primarily dissimilate organic compounds.

In recent years we have learned much concerning the mechanism of photosynthesis, while other autotrophic modes of life have received less attention than they deserve. Biologists can therefore be grateful to the Society for General Microbiology for sponsoring a symposium which treats autotrophy in the proper perspective: a fundamental biological problem, of which photosynthesis is only one specialized facet. Accordingly, the book deals with a number of fascinating bacteria which most contemporary textbooks either ignore or dispose of in a few highly misleading sentences.

The symposium opens with a paper by D. D. Woods and J. Lascelles, entitled "The No Man's Land between the Autotrophic and Heterotrophic Ways of Life," in which the general characters of various autotrophic

organisms are discussed. The authors make many illuminating comparisons and distinctions between organisms commonly classed as autotrophs and heterotrophs, emphasizing that no fundamental biochemical differences, apart from mechanisms of energy utilization, exist between the two classes. This chapter should therefore have been titled "Everyman's Land," rather than "No Man's Land."

K. A. Bisset and J. B. Grace point out that the major groups of autotrophic bacteria are pseudomonads or chlamydobacteria, while other autotrophs are probably colorless counterparts of blue-green algae. Their arguments are well taken and are independent of their contention that the pseudomonads represent a "primitive" group. This reviewer is disturbed by their attempts to locate *Thiovulum* among the protozoa.

The physiology of the Thiobacillus group is discussed by K. Baalsrud whose extensive experience with these organisms has enabled him to write an authoritative and informative chapter. Since the nitrifying bacteria are difficult to culture, less is known about them than about other autotrophic bacteria. It is therefore especially gratifying to find two chapters devoted to these organisms, one by J. Meiklejohn on their general physiology, and the other by H. Lees on their biochemistry. Dr. Meiklejohn's account, in addition to much valuable information, unfortunately also includes, uncritically, some references to work of questionable validity. Even more regrettable is her statement that autotrophs must be among the earliest forms of life. Biologists have for some time abandoned this notion, since it has become obvious that organisms which require only carbon dioxide to synthesize all organic matter are biochemically most complex and not primitive in any sense.

Nitrogen fixation and other aspects of nitrogen metabolism in the blue-green algae are the subject of a chapter by G. E. Fogg and M. Wolfe, followed by a chapter on nitrogen metabolism in the green algae by P. J. Syrett.

H. Gaffron has undertaken the difficult task of presenting present-day views on the mechanism of photosynthesis. In this chapter not only does he present the evidence that has been gathered from work on green plants and algae but also attempts to reconcile the various and often contradictory interpretations which investigators have put forth.

The experiments of H. Larsen have done much to revive interest in the green sulfur bacteria. His present account of the green and purple sulfur bacteria should go far in stimulating further work. Another aspect of the metabolism of purple bacteria, their ability to utilize organic compounds, is discussed by S. Elsden. He also includes some very recent experiments on the participation of cytochromes in the photo metabolism of these organisms.

Almost all photosynthetic organisms contain other pigments in addition to the chlorophylls. L. R. Blinks describes the rôle of some of these accessory pigments, phycobilins and carotenoids: some plants which possess them may greatly extend their spectral range of photosynthesis.

Our continuous search for new sources of energy and organic raw materials, prompted by the threatened exhaustion of fossil photosynthetic

products, has directed attention to autotrophic microörganisms as possible agents for the production of organic matter on a commercial scale. E. C. Wassink discusses the practical as well as fundamental problems encountered in the culture of algae on a mass scale. The difficulties which arise in attempts to produce dense populations in a short time provide, incidentally, an insight into the competition for light and space among photosynthetic plants in general. K. R. Butlin and J. R. Postgate conclude the symposium with a well-balanced survey of the economic importance of autotrophic organisms in general, including the activities of marine photosynthetic plankton on whose activities the entire fertility of the oceans depend. The authors justly criticize Rabinowitch for his statement to the effect that the activities of chemoautotrophs are "of no consequence" today.

Although, regrettably, almost no mention is made of hydrogen bacteria and *Clostridium aceticum*, the volume contains much of the information on autotrophic organisms available today. It is quite obvious, however, that we still know very little about the autotrophic bacteria and that much remains to be added to the studies which were so brilliantly initiated by Winogradsky in 1885.

Like the previous volumes of this series, the book is attractively bound and printed, but the lack of an index is a serious shortcoming.

WOLF VISHNIAC

THIRD ANNUAL REPORT ON STRESS. By Hans Selye and A. Horava. Montreal, Acta, Inc., 1953. 625 pp.

"Without generalization, there is no meaning and without completeness there is no significance" is a quotation from Alfred North Whitehead that appears on the title page of the third report of this series. This volume is complete in that it lists 5,741 new references (briefly annotated and arranged) according to subject. Subject groupings are based on a broad mixture of physiological, pathological and anatomical concepts (Parts I and II). The third part, "After-Thought, Sketch for Unified Theory of Medicine," supplies much of the generalization.

The references are complete with title, are listed alphabetically by authors, and include the progressive bibliographic numbers that are continued from the earlier volumes. The list requires 177 compact pages. It seems comprehensive and the basis of selection seems to be only that the title and content have some relationship to the broad field encompassed.

The introduction and a section of the first part of the volume define the scope and purpose of the book—"These books are obviously not meant to be read from cover to cover," and the method of using the volume "If you are in great haste, consult this book only through the "Subject Index—." Furthermore, this section gives definitions and presents the scope of *stress*. "Ever since man used the word 'disease' he had some, at least subconsciously, inkling of the stress concept." The scope is therefore very broad. The larger "Part II" contains the annotated, subject-classified references and three special reviews by invited contributors. The latter total 34 pages

and are on adrenalectomy in diabetes, neurophysiological control of ACTH secretion, and hypertension.

The volume is a unique type of bibliography arranged according to author and major and minor sub-groupings of the subject matter as well as listings of authors by serial numbers and may well serve as a model for other bibliographies. The critical reviews and comments add to the total bibliography in that they indicate to some extent the relative value given some of the references cited.

W. U. GARDNER

NERVE IMPULSE. Transactions of the Fourth Conference sponsored by the Josiah Macy, Jr., Foundation, March 4, 5, and 6, 1953. David Nachmansohn, Ed. New York, 1954. 224 pp. $4.00.

The receptor organs, which are the gate-keepers of one's sensorium, were for a long time the "forgotten men" of neurophysiology. The appearance of the present volume is another testimonial that they are now achieving "union" status. The book contains three presentations, the first by George Wald on the rods and cones of the eye, the next by Hallowell Davis on the receptor apparatus of the internal ear, and the last by Yngve Zotterman on thermal receptors. It brings together a great number of fascinating new developments that otherwise might be lost sight of for a long while in specialized journals.

Wald deals successively with the anatomy of the rods, the biochemistry of visual processes, and the *in vivo* and *in vitro* correlation of the adsorption spectra of rhodopsin and iodopsin. In conclusion he presents a model which suggests how one quantum of light, through the bleaching of one molecule of rhodopsin and the release of two to three sulfhydryl groups, might set off a chain reaction resulting in nervous excitation.

Davis, after a brief review of the anatomy of the inner ear, sets forth an intriguing explanation, backed up by compelling evidence, of how the energy of sound is transformed into electrical energy that in turn receives sufficient amplification to excite terminals of the cochlear nerve. In the process, he draws heavily on the beautifully executed experiments of Békésy, who appears to have achieved almost the penultimate in microscopic techniques. He concludes by showing records of action potentials from single fibers of the cochlear nerve which Tasaki recorded with glass micro-electrodes having an outside diameter of 0.2μ. Such are the technical advances that have at last made possible a refined analysis of receptor mechanisms.

As practically nothing is known about the anatomy of the thermal receptors, Zotterman is required to infer the nature of their activity on the basis of the impulses they trigger in the nerve fibers. He makes the notable observation that the "cold" and "warm" fibers indicate that the receptors continue to discharge at constant temperatures. This is contrary to the classical conception that receptors respond only to a *change* in the environment. The rate of the "stationary" discharge varies, but occurs within a predictable range, at different temperatures. He enlivens his presentation

by suggesting a number of interesting correlations between what is observed physiologically and what one experiences subjectively, e.g., paradoxical cold, persisting cold sensations, and so forth.

It is to be emphasized that the thermal fibers are of very small calibre. In winding up the discussion of his paper, Zotterman does the service of calling attention to the physiological significance of small fibers in general. Like the receptors, the small fibers have probably been neglected in the past because of the technical difficulties in investigating them. But being, as it were, out of sight, they have been out of mind, with the result that much more importance has been attached to the large, fast-conducting fibers whose large action potentials are much easier to record and to trace to their destination. This prevalent attitude was reflected in Zotterman's own thinking when he began to investigate depressor reflex mechanisms related to the heart and to the carotid sinus: "When we started working on them," he states, "we believed that the large afferent fibers from the right auricle were the sort of fibers subserving the real cardiac depressor effect, but," he continues, "we found out that they were not. The small delta fibers were the ones that were doing the trick. . . . It is only when the small fibers are stimulated [and this applies also to the carotid sinus] that we obtain the reflexes. . . . The small fibers have been overlooked before. . . . I found out that the small fibers are contributing much more to our sensations than the large fibers. . . . Of course, we have to look more carefully into the large fiber system and find out what they are doing centrally. The impulses reach the center much earlier than the small fiber impulses, but they are very few generally compared to the abundant mass of small fibers whose activity builds up the real emotions. The funny thing is that it is these tiny fibers which are giving us the emotions: their signalling is the basis of our emotional life."

It is to be hoped that the small fibers, like the receptors, will soon lose their status as "forgotten men." It is also to be hoped, as Chairman Merritt suggested in closing, that they will be the subject of one of the conferences on the nerve impulse in the near future.

PAUL D. MAC LEAN

ADMINISTRATIVE MEDICINE. Transactions of the First Conference sponsored by the Josiah Macy, Jr., Foundation, March 9, 10, and 11, 1953. George S. Stevenson, Ed. New York, 1953. 176 pp. $3.00.

This little volume, which should not be confused with the compendium on *Administrative Medicine* edited by Haven Emerson, will be of real interest to those concerned with policy problems in the health field, particularly the areas of professional education, hospitalization, and insurance. It furnishes a case report of attitudes toward current problems uppermost in the minds of a carefully selected group of participants most of whom carry responsibility for action at their own desks. In spite of the obvious limitations of translating conversation to the printed page, it does succeed to a remarkable degree in recreating the conversational tone. Since most of the participants are well known, many readers will find their reconstruction of

the conference aided by well-remembered tones of voice, mannerisms, and even gestures. The intentional lack of an agenda and the encouragement of free association resulted in an astonishing breadth of subject material, the encyclopedic nature of which afforded the faithful subject indexer a field day. Nevertheless, it was not intended that the list be all-inclusive, and it is not surprising that important areas were omitted though not necessarily overlooked.

There is a fascination in looking over one's own (and one's neighbor's) mending basket where all kinds of odd garments with interesting shapes and functions have a common resting place awaiting patching and darning. And yet this particular exercise has real value, because it realistically reflects the unresolved problems of the day. All in all this is a fascinating experiment in the art of communication focused on present problems. It has additional timeliness in view of the currently rediscovered emphasis on the values of intelligent conversation. It suggests itself as a welcome addition to the source material for the stimulation of graduate seminars on problems in the health field. Many will find this little book of interest. The careful reader is rewarded from time to time with nuggets of insight and illustration rarely found in the more formal presentations.

GEORGE B. DARLING

PROGRESS IN BIOPHYSICS AND BIOPHYSICAL CHEMISTRY. Vol. 4. J. A. V. Butler and J. T. Randall, Eds. New York, Academic Press, and London, Pergamon Press, 1954. 339 pp. $9.50.

The fourth volume in the series on *Progress in Biophysics and Biophysical Chemistry* lives up to the admirable standard set in the preceding volumes, both as regards the broadness of fields covered and the general excellence of the contributions. The chapters are:

1. Polyelectrolyte Gels by A. Katchalsky.
2. The Transference of the Muscle Energy in the Contraction Cycle by H. H. Weber and Hildegard Portzehl.
3. The Submicroscopic Structure of Nerve Fibers by H. Fernandez-Moran.
4. The Nucleoprotein Complex of the Cell Nucleus and its Reactions by P. F. Davison, B. C. Conway, and J. A. V. Butler.
5. Chromosome Breakage by P. C. Koller.
6. The Crystalline Proteins: Recent X-ray Studies and Structural Hypotheses by J. C. Kendrew.
7. Facts and Theories about Muscle by D. R. Wilkie.

The papers are written on a high scientific level and show signs of having been prepared with considerable thought. The book can be unhesitatingly recommended to all who consider themselves as biophysicists, as well as to many workers whose interests lie on the outskirts.

The price for the book seems definitely high, as though the publishers were anticipating purchase by libraries only, with negligible sales to individuals. This philosophy may be justifiable in regard to many of the annual reviews of scientific fields which appear these days. However, in biophysics

there are not available the textbooks and reference works summarizing the fundamentals of the field as are found in older and more established sciences. Consequently, this reviewer has found it profitable to keep his copies of *Progress in Biophysics* near at hand as a general reference work. The present volume is a worthy addition, but it is feared that the price will discourage many individuals from following a similar practice.

FRANKLIN HUTCHINSON

MICHAEL SERVETUS HUMANIST AND MARTYR. By John F. Fulton. With a Bibliography of his Works and Census of Known Copies by Madeline E. Stanton. New York, Herbert Reichner, 1953. 98 pp., illus. $8.50.

The life of Michael Servetus is a particularly fascinating one. This is due in part to his melodramatic indiscretions inevitably leading to a tragically dramatic climax, in part to the startling significance of his relatively slight literary production, and, indeed, in part to the fact that we know so little about his life. Therefore any serious effort to contribute to our knowledge of the life and career of Servetus is very welcome. The present volume begins with a study of Servetus in which Dr. Fulton, while considering his entire life and martyrdom, has chosen to emphasize the medical career and especially the rôle of Servetus in the discovery of the circulation of the blood. This account begins quite properly, therefore, with a consideration of Galen, ultimately recognized as having contributed in some degree to the idea of the circulation but immediately an obstacle through his notion of the permeable septum of the heart. This was the fundamental Galenical error which held sway for well over a thousand years until first doubted by Vesalius in 1543 and fully denied in 1555. Between these dates Servetus almost casually described the alternate route of the blood through the lungs in several pages of his major theological treatise, the *Restitutio Christianismi*, first in a manuscript draft of *c.*1546 and thereafter in the published version of 1553. The *Restitutio* is a symbol of the career of Servetus since it begins with theology, briefly turns to medicine before reverting to the first theme, and, like its author, was finally silenced by a destruction so thorough that the description of the pulmonary circulation contained in it appears to have remained unnoticed for nearly a century and a half.

The relatively short medical training that Servetus received (at least insofar as our evidence indicates), the fact that his major interest in his final years was theology, and the location of the description of the pulmonary circulation in a theological work, have led many to wonder how this man, presumably ill-equipped medically, could have achieved a discovery of such vast importance. Dr. Fulton indicates several facts which go far to explain this problem. Servetus was a Galenist and while there is much in Galen which opposes the idea of the circulation, there is also much which might lead to an intuitive revelation. This presumes a certain grounding in anatomy, and Jacobus Sylvius, as Dr. Fulton points out, mentioned only two of his students as his outstanding assistants in anatomy. The fact that Vesalius was one gives us some idea of the ability of Servetus as the

other. Nor should those years during which Servetus practised medicine in Charlieu and Vienne be overlooked. Certainly he must have been much preoccupied with bloodletting and possibly—although we have no evidence for it—with anatomical research. In recent times it has been pointed out that much earlier a Persian physician, Ibn an-Nafis, had described the pulmonary circulation, and some effort has been made to demonstrate that Servetus was capable of reading Arabic and may have been influenced by this earlier account. There is, however, no sound evidence for his knowledge of Arabic while, on the other hand, the other descriptions of the pulmonary circulation which follow hard upon that of Servetus indicate that the discovery was in the air and sooner or later someone would grasp it.

As with many great discoveries, several persons came upon this one at about the same time. The result was unfortunate since this fact has led to some rivalry, at least in part directed by nationalism, for precedence in the discovery. While there is no doubt of the written and printed priority of Servetus in the western world, first in *c.*1546 and thereafter in 1553, yet close upon this latter date occurs the description by Juan Valverde de Hamusco in 1556 and that of Realdo Colombo in 1559. In the two latter instances the descriptions are prefaced by the remark that it was something hitherto unknown to anyone. However, this expression was used by sixteenth century writers with such frequency as to become meaningless. Dr. Fulton calls attention to the remarks of Dr. Josep Trueta who has sought to prove that both Valverde and Colombo took their information from a copy of the *Restitutio* but did not acknowledge this source since the book had been condemned as heretical, and in part Dr. Trueta seeks to support this view with the statement that Valverde's *De Animi et Corporis Sanitate*, published in 1552, makes no mention of the circulation. There is, of course, no proof for the first portion of this statement and the title of Valverde's book of 1552 is such that one would not expect to find the description there. Colombo's book was published posthumously in 1559 with the complicating statement that it had actually been many years in preparation, and certain Italian writers have made use of this to support a priority of discovery for Colombo. On the other hand, Trueta as the defender of Servetus has sought to show that Colombo's account is based on the description of Servetus, but any similarity—if it did exist—like a two-edged sword could well cut in either direction. Dr. Fulton has meticulously presented these complicating factors but wisely gives his allegiance to none of them, since, in the final analysis, we must give support to substantial evidence rather than to wishful thinking and unsupported theories. Who first may have had the idea of the pulmonary circulation is unknown. The idea was in the air, and it was natural that it would be grasped by several at about the same time. However, Servetus' priority is supported by the manuscript of *c.*1546 and the published version of the *Restitutio* of 1553.

Not the least mysterious aspect of the life of Servetus has related to his writings. What precisely did he write, where and how frequently were these writings republished, how common or rare are they today and where are the surviving copies of them presently located? The answers to these questions will tell us a considerable amount about Servetus, the extent of his

popularity and influence in his own day as well as later, lay certain legends which have persisted, and assist the modern scholar to locate these tools of his research. For all of these reasons the present bibliography of the writings of Servetus is very valuable indeed.

It has long been assumed that the first two tracts, *De Trinitatis Erroribus,* 1531, and *Dialogi de Trinitate,* 1532, are exceedingly rare. Indeed, it was because of this assumed rarity that the counterfeit editions of 1721? were published. Now we find that this is not the case and that they are well represented in European and American libraries. A second troublesome matter has been the question of the editions of the *Syruporum Universa Ratio.* It may now be held with considerable assurance that there are four sixteenth century editions of which copies are definitely known to exist in libraries. Regrettably no further copies of the *Restitutio* have been discovered, but the bibliographical descriptions of the three surviving copies, conveniently brought together and summarized are of no little value, and the locations of copies of the 1790 reprint in American libraries indicate that it remains a book of considerable rarity on this side of the Atlantic. Some indication of the interest in Servetus which has continued from the seventeenth century, when he was in a sense rediscovered, down to the present is to be gained from the wide distribution of his various writings as well as the high prices commanded by his works even as early as the late eighteenth century. The bibliography is very generously assisted by the inclusion of illustrations of all the title-pages, the pages from the Paris copy of the *Restitutio* which contain the essential part of the description of the pulmonary circulation, and a page of the manuscript of that work, also in Paris.

The book, limited to an edition of 750 copies, is a handsome one, and both the biographical essay by Dr. Fulton and the bibliography by Madeline E. Stanton, well-integrated one with the other, are to be warmly recommended to everyone who has an interest in Servetus and all that he represents, the question of the circulation of the blood, and generally a concern with bibliophilism and bibliography, truly a wide range of interests.

CHARLES DONALD O'MALLEY

EVENTS

PUBLIC HEALTH SEMINAR

October 18, 1954

MODERN PROGRESS OF PUBLIC HEALTH, FOOD TECHNOLOGY AND LAW AND THE SCIENCES OF NUTRITION IN SWEDEN AND WESTERN CONTINENTAL EUROPE GENERALLY. By Ernst Leopold Abramson, Director of the National Institute of Public Health in Sweden.

Sweden is occupied by a culturally homogeneous population of 7,000,000, 66% of which lives in densely populated regions distributed throughout an area the size of California. In 1953 a death rate of 9.7/100,000 obtained, with an average life expectancy of 69 years for males and 71.6 years for females. Cardiovascular disease and tumors account for 49.5% of all deaths, the high incidence of these diseases being attributed variously to longevity, skilled diagnosis, or a real increase over previous years.

The population is generally well fed, although the diet is moderately high in fat, and an attempt is being made to correlate the incidence of cardiovascular disease with this high fat intake.

The National Institute of Public Health, founded in 1938 with aid from the Rockefeller Foundation, serves in an advisory capacity to both government agencies and private persons. It is composed of four departments embracing the fields of public, industrial, and food hygiene and vitamin research.

Most medical care is provided by the government. Hospital care costs $10.60/day and three-fourths of all medical bills are paid by the state. This extensive welfare program is ultimately financed by money obtained from public taxation and special levies. Expectant mothers go to maternal and infant care centers, where they are confined free of charge and are given advice on birth control and infant care. Free medicine and prophylaxis are provided the child until he reaches school age, when his health becomes regulated through the school. One free meal each day is provided for school children. Dental care is also offered until the age of 15 years, and progress is being made in the control of ubiquitous dental caries through research on sugar consumption in the form of sweets and consideration of problems in the fluoridation of water stores. Each province has one or more dispensaries devoted to tuberculosis control including vaccine administration and mass x-ray.

It is interesting that although the Swedish government plays the major rôle in the administration of medical care and subsidization of research, nevertheless, academic investigation and medical practice are relatively free from bureaucratic interference, their function being happily extra-political as evidenced by non-partisan unanimity in the formation of public health policy.

E. D. C.

ZOOLOGICAL JOURNAL CLUB

October 27, 1954

RECENT STUDIES ON EMBRYONIC INDUCTION. By Dr. M. C. Niu, School of Biologic Sciences, Stanford University, California.

We began our investigation into the chemical control of ectoderm differentiation because we thought that it would be more fun than the studies on pigmentation that we had been pursuing for the previous twenty years. Embryonic induction was already one of the most studied fields in embryology, but no clear principles had been enunciated. The only real conclusion to come from earlier work was that the inductors could have an effect only when in direct contact with the ectoderm. Thus, it was concluded that diffusible substances were probably not involved. Our work proved this incorrect.

In a drop of nutrient medium (with a pH of 7.8, and a phosphate buffer, and lacking in Mg^{H}) we placed a small piece of ectoderm (5-8 cells, from a stage 10 newt embryo) and a larger piece of inductor tissue (chorda mesoderm, nerve plate, somite, etc.). The two tissues were never in contact. Subsequent incubation showed that the differentiation undergone by the ectoderm was dependent upon age of the inducing tissue. At one stage the ectoderm would be induced to form nerve and pigment cells and at a later point, myoblasts. Ectoderm which was incubated in medium in which an inductor tissue had previously been grown, though all traces of the latter were now removed, would also undergo differentiation into nerve cells. If this same inductor were permitted to continue its growth in fresh medium, and this medium were then drawn off and used to incubate another piece of ectoderm, a greater number of myoblasts and fewer nerve cells would differentiate. A third drop of fresh medium might now be added to the same inductor, again drawn off, and this time the ectoderm incubated in it would form an even higher proportion of myoblasts. Thus, we conclude that (a) inductor tissue can release diffusible substances capable of acting at a distance, (b) there exists a qualitative difference in the type of differentiation as the age of the inductor changes. Further experiments also indicated a qualitative difference between nerve plate and somite material when used as inducers. (The ectoderm used always came from the same site.)

Chemical analysis showed that the substances responsible for differentiation were either RNA, DNA, or both. Nucleic acids supplied by laboratories other than our own had no effect, although our own preparations did have some inductor effects, the degree varying with the source of the nucleic acids. The problem remains, of course, as to why differentiation will vary with the age of the inductor.

P. K.

ZOOLOGICAL JOURNAL CLUB—WOODWARD LECTURE

December 8, 1954

INNATE RELEASING MECHANISMS AND THEIR RELATION TO IMPRINTING. By Konrad Lorenz, Max-Planck Institut, Buldern, Germany.

It has long been known to zoo keepers that tame birds cannot be bred. This is due to their courting humans rather than members of their own species. Such a transference of sexual attention to abnormal objects is due to impressions received early in ontogeny. This type of learning, or imprinting, is characterized by being limited to a highly specific period of sensitiv-

ity, irreversibility, and fixation at a time prior to the operation of the function (e.g., sexual fixation occurs long before sexual maturity is attained). Hitherto, it has been demonstrated only among birds, insects, and certain fish.

All the reactions of an animal are not related to one particular object of fixation. Thus, a jackdaw which I raised by hand regarded me as its parent, accepted hooded crows as its traveling companions, courted my mother's maid, and adopted a young jackdaw as its young! The questions raised by such behavior include the factors that determine the fixating stimulus (Greylag geese reared by foster parents still exhibit normal sexual behavior, unlike Muscovy ducks or many other species) and the time of the sensitive period (the jackdaw-following reaction can be fixated on humans at a stage when the sexual reaction is no longer susceptible to imprinting).

Imprinting may have permanent effects on behavior which act very much like genetic traits, e.g., the host preferences of parasitic wasps. Thus, habitat selection, at least in insects, may be influenced by imprinting. No true imprinting is known, as yet, in mammals. This may be due to their sense of smell and ability to perceive their selves. In the case of humans, it would be of interest to review the objects of fixation to determine the degree of similarity that exists in the period during which the fixation occurred. It may be that insufficient efforts have been made to study this phenomenon in mammals and humans.

P. K.

YALE MEDICAL SOCIETY

October 11, 1954

THE CHEMISTRY OF COENZYMES. By Dr. John Baddiley, Lister Institute, London, England.

The mode of action of the vitamins, the enzymatic catalysts for many of the body's essential chemical processes, is indeed perplexing. Almost nothing is known of the action of the fat soluble vitamins A, D, E, and K; and the water soluble vitamin C. It is with the "B" vitamins that research has been most fruitful.

The "B" vitamins apparently function as coenzymes by uniting with a phosphorus-containing complex which, for many of the "B" vitamins, consists of adenosine dipyrophosphate. The exact function of the adenosine complex is not known. Investigation has shown that this complex is not involved in the transfer of metabolites from the substrate. Possibly it provides the linkage between the coenzyme and the apoenzyme.

Riboflavin links with adenosine dipyrophosphate to form the coenzyme portion of the flavoproteins, or yellow enzymes, which are active as dehydrogenases. The enzymes formed from nicotinic acid, diphosphopyridine nucleotide (DPN or coenzyme I) and triphosphopyridine nucleotide (TPN or coenzyme II), are also dehydrogenases. Finally, it has been recently shown that pantothenic acid links with this adenosine complex and with mercaptoethylamine to form a coenzyme (coenzyme A) which functions in acetylation. Coenzyme A is essential for all living cells as it has already

been implicated in citrate metabolism, long chain fatty acid synthesis, steroid synthesis, and porphyrin synthesis.

G. MC K.

YALE MEDICAL SOCIETY

November 8, 1954

THE RÔLE OF INTRAOCULAR PRESSURE IN THE GROWTH OF THE VERTEBRATE EYE. By Alfred J. Coulombre, Department of Anatomy.

In a previous study of the histogenesis of the chick retina, several observations suggested that intraocular pressure plays a cardinal rôle in the regulation of eye size during development. To test this possibility, short lengths of microcapillary glass tubing were introduced into the vitreous body through the walls of the eyes of forty-nine chicks at four days of incubation. Such tubes allowed egress of vitreous humor and relieved intraocular pressure. Controls were operated in exactly the same way except that solid glass rods were employed instead of tubes so that vitreous humor did not escape. The equatorial diameter of operated eyes was determined at daily intervals for four days following operation. The eyes with tubes showed virtually no increase in size over the four-day experimental period, whereas unoperated contralateral eyes and eyes into which solid glass rods had been placed showed the sevenfold increase in diameter normal for this developmental period.

It was noted that, in tube-operated eyes, the lens and cornea failed to grow, that the pigmented epithelium (in which mitosis ceases at four days of incubation) failed to increase in area, and that the neural retina (to which new cells are added until the fifteenth day) continued to increase in area and was thrown into folds.

The results suggest that intraocular pressure is a cardinal factor in the growth of the eye, and that it may be a common factor underlying the remarkable temporal correlations in the growth phases of the various components of the eye.

ALFRD J. COULOMBRE

SPLENECTOMY IN COOLEY'S ANEMIA. By David H. Clement, Department of Pediatrics.

Although Mediterranean (Cooley's) anemia is generally classified with the hemolytic anemias, it is fundamentally due to a racial, inborn error of metabolism characterized by inadequate synthesis of hemoglobin. It is not surprising that in the past splenectomy has been followed by failures. As the disease progresses, however, marked enlargement of liver and spleen develops and concomitantly the patient's transfusion need greatly increases.

Recent investigations have shown that normal blood has a shortened survival time in such patients. An extracorpuscular defect supervenes as the disease progresses.

Four patients with severe Mediterranean anemia with marked splenomegaly and large transfusion requirements were splenectomized. In all, the post-operative need for blood was reduced 25-50% of the pre-

operative level necessary to maintain a hemoglobin of 6 Gm.%. The children also showed striking improvement in disposition, appetite, and activity. No complications were encountered in the removal of these huge spleens or in the subsequent one to ten years of follow-up. Among the benefits of the procedure are the longer intervals between transfusions and the reduced amount of iron (blood) injected into these patients whose iron overload in the form of hemachromatosis is already a serious part of their disease.

DAVID H. CLEMENT

ELECTRON MICROSCOPE STUDY OF TISSUE CULTURES INFECTED WITH HERPES-B VIRUS. By M. Reissig and J. L. Melnick, Department of Preventive Medicine.

A time-sequence has been made of the morphological changes occurring after herpes-B infection as revealed in ultrathin sections under the electron microscope. Monolayer cultures of monkey kidney epithelial cells were infected with herpes-B virus, the simian member of the herpes group. The resulting cellular alterations were correlated with the appearance of infective virus in the culture fluids.

Morphological changes consist of the swelling of the cells and disappearance of the nucleolus, followed by margination and gradual decrease of the nuclear chromatin. The inclusion material corresponds to the clear central areas of the nucleus where the chromatin has disappeared. In the late stages of infection this inclusion material fills the nucleus and forms a classical type A inclusion body.

Characteristic particles appear in the nucleus and the cytoplasm of the infected cells a few hours after inoculation. They are formed by a dense center surrounded by either one or two membranes. Those with one membrane range in size from 60 to 100 millimicrons and those with two membranes, from 120 to 180 millimicrons. Particles showing the same wide variation in size and structure are seen both in the nucleus and in the cytoplasm. They are first seen on the external surface of the swollen but intact cells at about the same time that new infective virus becomes detectable in the culture fluid.

M. REISSIG

CHEMISTRY COLLOQUIUM

October 22, 1954

PHYSICAL CHEMISTRY OF NUCLEIC ACIDS. By Paul Doty, Harvard University.

It is commonly known that the structure of DNA is sustained by the combination of the four bases—adenine, thymine, guanine, and cytosine—with desoxyribose and phosphoric acid in the formation of a complex macromolecule. By the use of x-rays, light scattering and viscosity measurements it has been possible to obtain a notion of some of the physical properties of this biologically important substance. Although the molecular weight of DNA is about 6,000,000, its end-to-end length is only 5000 Å and its

contour length, 20,000 to 30,000 Å. From these measurements it is apparent that the spatial configuration of the molecule must be that of two chains arranged side by side. This conclusion is supported by x-ray diffraction patterns which indicate a helical arrangement. A cross-section through such a structure would demonstrate a concentric arrangement of base, phosphate, and hydrogen bonds. An oversimplified model of such a molecule may be had by imagining a house painter's ladder made of rubber and twisted a number of times about its longitudinal axis. In this case the rungs of the ladder represent hydrogen bonds, while the two sides represent strings of base and phosphate. Such a conception is consonant with the theories of classical genetics.

If dilute solutions of DNA are titrated with acid or alkali, it is found that outside the limits of pH 2.6-11.9 the molecular weight of the substance decreases, indicating dissolution of its physical structure. However, between these limits the molecular weight remains constant, although there is a change in spatial configuration as demonstrated by light-scattering phenomena. Under these circumstances, should the DNA be ultracentrifuged, it can be demonstrated that its intrinsic viscosity has fallen, while its sedimentation constant has remained unchanged. Within certain time limits this change in intrinsic viscosity may be reversed. Identical behavior of the DNA molecule occurs in heat denaturation. From these observations it has been postulated that the molecule, while retaining its structural integrity, collapses spatially to a considerable extent and thus succeeds in immobilizing enough solvent to cause a measurable change in its intrinsic viscosity.

In subjecting the DNA to enzymatic degradation there is an initial latent period in which molecular weight falls very slowly or not at all. There is, however, no corresponding latent period in the curve representing change of biologic activity, which falls immediately upon initiation of enzymatic degradation. If the molecule is degraded by acid titration beyond the limits of reversibility, there is no levelling off of the molecular weight curve as might be expected if the degradation served to separate two previously linked chains. For these reasons it is assumed that the enzyme attacks chemical bonds which are disposed along the longitudinal axis of the molecule. The immediate interruption of some of these bonds results in a reduction of biological activity; but with the dissolution of two bonds situated at points opposite each other along the chain, the macromolecule is severed and the molecular weight is observed to decrease.

E. D. C.

MICROBIOLOGY SEMINAR

October 29, 1954

TRI-CARBOXYLIC ACID CYCLE IN ESCHERICHIA COLI. By Charles Gilvarg, Department of Biochemistry, New York University School of Medicine, New York.

The tri-carboxylic acid cycle has been studied extensively in muscle and other animal tissues but its rôle in bacterial metabolism has been neg-

lected. From the distribution of isotopically labelled glutamic and aspartic acids and the identification of specific enzymes, it was thought that the cycle could function in *E. coli*, but there was some question concerning the impermeability of the cell to alpha-ketoglutarate and citrate subsequent to metabolic block with malonate. The use of the proper micro-organism has overcome this objection.

By careful selection for a strain of *E. coli* which requires glutamic acid, a mutant, M2264, has been isolated in which alpha-ketoglutarate can replace the glutamic acid. The metabolic block rests in the inability to synthesize citrate—hence acetate cannot be used as a carbon source. This is not a double mutant since all irradiated reversions to the wild type resumed the capacity of using acetate.

The defect in using acetate was evaluated more closely in the Warburg apparatus. There was no apparent oxidation of acetate by the mutant and when endogenous respiration was determined from CO_2 measurements, only 0.19% of the labelled acetate was converted to CO_2 contrasted to an 80% conversion by the wild type. Since the ability to grow on acetate depends upon the presence of the condensing enzyme, the mutant, lacking the enzyme, had only 1/400 of the ability to oxidize the acetate. In this organism, the cycle functions as the only means of developing the carbon skeleton of glutamic acid and the interruption of the cycle results in an absolute requirement for alpha ketoglutarate.

M.-L. J.

MICROBIOLOGY SEMINAR

November 5, 1954

THE PHYSIOLOGY AND BIOCHEMISTRY OF PLANT DISEASES. By David Davis, Connecticut State Agricultural Experiment Station, New Haven, Connecticut.

In a study of Fusarium wilt of tomatoes, the etiologic agent, *Fusarium glycoperside*, has been maintained on an artificial medium of glucose and inorganic salts. The fungus, which has no sexual stage and grows by budding, is a common soil contaminant which attacks the vascular elements of plants, killing either by mechanical plugging or by a toxin and subsequent host-parasite reaction. Actually Fusarium is known to produce two noxious substances, one a polypeptide and the other a pyridine-like compound, both of which when isolated can produce wilt. However, many other things, including finely ground normal tomato stem, will lead to wilt as well as vascular discoloration. This has seriously handicapped the attempt to demonstrate a toxin in tomato plants infected with Fusarium. With the technique of grafting healthy plants of the same or another species to infected tomato plants, the fungus can be seen to be localized in the stock whereas the toxic effects are apparent in the graft.

The symptoms described for Fusarium wilt can be reproduced by various chemical substances. Epinasty, for example, results from ethylene poisoning while phenolic substances will contribute to vascular disease and in the

presence of phenol oxidase yield a characteristic discoloration. When plants are infected with Fusarium, phenolic acid reactive with phenol oxidase can be detected in two to three weeks, the phenols having been stored as beta-glucosides. The breakdown of beta-glucosides as well as tannins and lignins leads to discoloration. The symptoms of vascular wilt can be demonstrated when natural pectins are in contact with hydrolyzing enzymes and a gel is formed which clots vessels. Pectin methyl esterase produced by Fusarium is noted to hydrolyze pectins more rapidly in the diseased plant as contrasted to the healthy one and a polygalactinase is also elaborated by Fusarium if the proper substrate is present.

M.-L. J.

ANATOMY SEMINAR

November 16, 1954

INTERACTION OF STEROIDS ON REPRODUCTIVE MECHANISMS. By Dr. Joseph Velardo, Harvard University.

In the female rat, cervical stimulation followed by uterine traumatization results in the "decidual reaction," which is the experimental equivalent of the formation of the maternal placenta. Ovariectomy abolishes this response, though it may be reëstablished through the subcutaneous injection of progesterone. By injecting other steroids along with progesterone, a quantitative measure of the effect of the interaction of these substances on the deciduomata can be obtained. In this manner the following results were obtained.

Of the estrogens, estradiol proved the most effective of the progesterone antagonists. ACTH and testosterone acted similarly. Discovery of the antagonism between progesterone and pregnandiol proved perplexing, until further work indicated that it is synthetic isomers of pregnandiol, and not the naturally occurring product, that act in this manner. Pregnane 3-beta-20-beta diol was a particularly good inhibitor among this series. Pregnandiones from different pharmaceutical houses were also shown to have different inhibiting potencies, which underscores the need for determining the exact source and nature of biochemical preparations.

A second response-indicator, which was used for other experiments, involved the administration of estradiol to castrated female rats. The estradiol effectively promoted uterine growth, which was then taken as an index of the degree of interaction of the various steroids. Estrone, administered in place of estradiol, also proved potent in promoting uterine growth, though less so, while estriol had low potency. Since all three estrogens occur together in normal animals, various combinations were tested. Estriol, it was thereupon found, depressed the activity of both the other compounds. The effect of cortical steroids, DOCA, testosterone, and cortisone, was also investigated. It was suggested that the interactions might be due to competition between molecules for reaction sites.

P. K.

ARTHRITIS STUDY UNIT

November 18, 1954

THE PRINCIPLES UNDERLYING THE MANAGEMENT OF OSTEOPOROSIS. By Ephraim Shorr, New York Hospital, New York.

The frequent occurrence of osteoporosis secondary to prolonged courses of Cortisone therapy has focussed attention on the proper management of this perplexing clinical problem. For some time it has been known that strontium, a mineral closely related to calcium in the periodic table, is deposited in the body in a manner similar to calcium. Further, it has been shown that a patient will continue to deposit strontium after a "ceiling" of calcium deposition has been reached. With these facts in mind, balance studies were performed on several patients with osteoporosis of various etiologies. In almost all cases, balances which had been negative for calcium became positive for calcium plus strontium when the patients received diets containing 1.5 grams of calcium and 1.5 grams of strontium per day. These changes were accompanied by improvement in the osteoporosis as judged by the x-ray. These positive results could be augmented by the use of one, or all of the following: Estrogen (10,000 units/day), Testosterone proprionate (25 milligrams/day), and vitamin D (50,000 units/day). However, patients on very high dosages of Cortisone continued to show negative calcium balances, despite the most intensive therapy, until the dosages of Cortisone were reduced.

G. MC K.

WOODWARD LECTURE

November 18, 1954

THE CHEMICAL STRUCTURE OF NUCLEIC ACIDS. By Sir Alexander Todd, Professor of Organic Chemistry, Cambridge University.

Following initial studies on the purine and pyridine bases and the sugars found in nucleic acids, investigation was turned to the synthesis of the larger structural units, the nucleotides, consisting of base, pentose, and phosphate. It had long been recognized that nucleic acids consist of chains of such units linked through phosphate, but the location of the phosphate bridge between pentose units of ribonucleic acids (RNA) had remained uncertain. Fresh evidence on this question came with the chromatographic demonstration by Carter and Cohn in 1950 that the products of alkaline hydrolysis of RNA consisted not, as had been thought, of four nucleotides, but of eight, comprising two isomeric derivatives of each base. These were designated *a* and *b* and were shown to correspond to the synthetic 2′- and 3′-phosphates of the nucleotides, but which was 2′- and which was 3′- was not known. Either form, on standing in weak acid, gave rise to an equilibrium mixture of the two, and this was interpreted, by analogy with the known behavior of other similar structures, as occurring through a cyclic 2′-, 3′-monophosphate intermediate. This provided an explanation for the lability

of RNA in acid and alkali; such cyclization occurring within a polynucleotide chain would produce an unstable structure in which the bond to the adjacent nucleotide would break.

That such cyclic intermediates are indeed formed during the hydrolysis of RNA was established by Markham and Smith's isolation of them. The well-known stability of deoxyribonucleic acids is then due to the 2′-position of their sugar (2′-deoxyribose) being available for cyclization.

The isolation by Cohn in 1951 of 5′-nucleotides from enzymic digests of RNA proved that this position is implicated in the internucleotide linkage. This linkage must therefore have been either 2′—5′ or 3′—5′. The decision between these possibilities came from studies of the action of ribonuclease. This enzyme was shown to attach a second phosphoester bond on pyrimidine *b* nucleotides, but had no effect on derivatives of pyrimidine *a* nucleotides, nor of any purine nucleotides. Since RNA is hydrolyzed by the enzyme, the structure in RNA must be of the *b* form. It remained only to determine whether *b* represented 2′ or 3′. This was done by an unequivocal synthesis of 2′-adenylic acid, which was found to correspond to adenylic acid *a*. The *b* position is therefore the 3′, and the internucleotide linkage must be 3′—5′. This proved for RNA a structure analogous to that of DNA, in which 3′ and 5′ are the only positions open to esterification.

A question still not settled is whether RNA is composed of simple or branched chains. There is only one way in which branches are possible: through phosphate from a 2′ position in the main chain to a 3′ position in the side chain, for triply esterified phosphate would be too unstable, and linkage to the 5′-position in the side chain would give a structure not labile to alkali.

The following technique might be used to study the question of branching or the sequence of bases in RNA. The terminal ribose unit, having both 2′- and 3′-hydroxyls free, contains a *cis*-glycol configuration which may be oxidized with periodate, liberating the terminal base (with an aldehyde fragment which may be removed with alkali too weak to touch the rest of the chain. After removal of the newly exposed phosphate with monoesterase, the process may be repeated, thus degrading the chain in a stepwise manner until a branching point, where there is no *cis*-glycol structure, is reached. Application of this method awaits the preparation of a homogenous ribonucleic acid.

E. D. C.

WOODWARD LECTURE

November 29, 1954

EVOLUTION AND HUMAN DESTINY. By Julian Huxley, London, England.

Religions, essentially, are organs of man in society which enable him to deal with the problems of his destiny. Humanism, too, could be the basis for a religion, for it is similarly focussed on the question of man's significance in the universe. There are other religions which are equally nontheistic

(e.g., Buddhism) or nondoctrinaire (e.g., Hinduism). And all of them, including humanism, involve cognitive, moral, and ritualistic aspects. Of these, the cognitive assume the greatest importance, for what one thinks about nature and man's place in it will largely shape one's morality and rituals. Only now, however, has science begun to make possible the replacement of religious myths with scientific concepts so that the cognitive aspect can be based on facts.

The advance of science in the past three centuries has progressively limited the need for the postulation of Divine Intervention to explain nature. Newton introduced the idea of a universal motion, and, by the nineteenth century, the theories of Darwin made man a part of nature. At this time was marked the beginnings of the sciences of man—anthropology, psychology, sociology, *et alii*. Finally, science's interest is now switching from the actual to the possible, from the "Descent of Man" to the Ascent of Man.

In the meantime, the established religions of the world have reached a crisis, losing their hold on individuals as on political institutions. Their doctrines, though purported to represent absolute truths are neither mutually compatible nor consonant with the facts of nature which we now know. Finally, they are dualistic, while scientific thought is increasingly favoring monism. Thus, we see the rise of the religions of Christian Science, Naziism, Marxism, and Existentialism, which attempt to correct this dualism and disconsonance. The humanistic movements (Unitarianism, Ethical Culture, etc.) attempt to do the same, but suffer from a lack of an agreed-upon picture of man in his setting, a rarefied morality, and an optimism that takes no cognizance of sin.

We can now see all of reality as a single process, that of evolution. Evolution falls into three main phases, each with its own characteristics, the inorganic, organic, and human. Apparently, all possible physical forms had been exhausted by the Pliocene. That man was subsequently able to rise to dominance was because of his mental, rather than his physical, attributes: the reproducibility of the products of the mind had become possible as the human phase developed from the organic. This enabled evolution to proceed with ever-increasing velocity.

Only man's intellectual capacities, ultimately, are decisive. Present knowledge will doubtless lead to new views on morality and religion. Science's main contribution is in the fact that Truth must be progressively discovered and that one must work to make Truth prevail.

Man's place in nature is in the vanguard of evolution on earth, his rôle is to be the agent or instrument of evolution in the future, and to make the evolutionary process transcend itself. For this he has ample time. This planet can probably continue to exist for much longer than it already has.

This is a new picture of man's destiny—one based on science—and this makes it possible for science and religion to join hands in the new humanism.

P. K.

BIOCHEMISTRY SEMINAR

December 7, 1954

RECENT ADVANCES IN BIOCHEMISTRY OF INSECT GROWTH AND METABOLISM. By Carroll Williams, The Biological Laboratories, Harvard University.

Study of metamorphosis of the giant silkworm, *Platysamia cercropia,* has pointed to the existence of a new addition to the cytochrome system. This factor, cytochrome b_5, present in all non-muscular tissue tested, has perhaps escaped previous notice in mammalian tissue because its absorption maximum, 560 mμ, may have been masked by that of reduced hemoglobin.

After pupation the insects enter a non-developing pupal diapause of minimal metabolic activity. Time of incubation before emergence from diapause is reduced from fifty-two to three weeks by chilling at 5° for 10 weeks and then returning to room temperature. Chilling is shown to stimulate secretion of hormone from neurosecretory cells in the brain and this, in turn, activates the secretion of another hormone from the prothoracic gland. This latter growth and differentiation hormone is active *in vivo,* stimulating all phases of insect growth and, *in vitro,* inducing spermatogenesis from isolated testis. Growth and differentiation hormone has been purified in crystalline form by Butenandt and collaborators in Germany revealing the empirical formula: $C_{18}H_{30}O_4$ (insol. H_2O; sol. methanol; m.p. 235-237; ultraviolet max. 249 mμ; infra red max. 6.12 μ).

The minimal metabolism of pupal diapause is unaffected by cyanide; larval and pupal stages, however, show normal sensitivity. CO acts similarly and is reversed by light, indicating the action is on cytochrome oxidase. The non-sensitive respiration is attributed to cytochrome b_5. Quantitative studies show non-muscular tissues contain cytochromes b, b_5, c, and oxidase in larval and adult stages, but only b_5 and oxidase are measurable in the pupa. Respiration in diapause is therefore probably by direct transfer from cytochrome b to oxygen.

The increment of growth and differentiation hormone terminating diapause parallels the reappearance of cytochrome c. It is the working hypothesis that this hormone stimulates cytochrome c synthesis to provide the extra respiration necessary for development.

RICHARD I. BREUER

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Kodak reports to laboratories on:

Our sadness about a new reagent for ascorbic acid... telling your story without jump or jerk... investigating photoelasticity

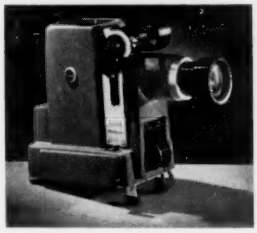
Vitamin C test

A new and apparently improved colorimetric assay method for ascorbic acid has been published recently and we're a little sad about it. Our sadness stems from the fact that the reagent widely favored hitherto for ascorbic acid has been the redox indicator *2,6-Dichloroindophenol Sodium Salt* (Eastman 3463). We know a trick that gives us a *2,6-Dichloroindophenol Sodium Salt*, of which we are exceedingly proud. Now along come some people with an entirely different method, based on the enediol character of ascorbic acid rather than on its reducing proclivities. They claim that dehydroascorbic acid does not interfere in their test. (Reduce it to ascorbic acid with H_2S if you want it.) They further claim no interference from anything else likely to occur in finished pharmaceutical products, natural juices, or processed foods. They say sensitivity is 10 micrograms of ascorbic acid per ml. We have no reason to doubt them. They suspect the method works for biological fluids also and promise to look into that soon. Our only solace is the knowledge that the reagent for the *new* method is *4-Methoxy-2-nitroaniline* and that this too, in most satisfactory quality, has been in our catalog for many years as Eastman 2094.

The way to get a free abstract of the new procedure is to write Eastman Organic Chemicals Department, Distillation Products Industries, Rochester 3, N. Y. (Division of Eastman Kodak Company). You can even place your order at the same time for some 4-Methoxy-2-nitroaniline *if you're impatient to try it out. 25 grams for $1.50, 100 grams for $4.50.*

Talking with film

There is a man in your trading area who calls himself an audio-visual dealer. This mid-20th-century addition to the roster of trades is a merchant of various devices for captivating a captive audience into granting access for your message to their minds through their eyes and ears. Assuming you have a message—most successful people nowadays have one—we point here to a new and advanced example of one of the basic devices which your audio-visual man has for



conveying it, the *Kodaslide Signet 500 Projector, Filmstrip Model.*

First we ask you to grant that the smoother the mechanical aspects of your presentation, the less likely it is to distract your audience from your subject matter. Therefore we have proceeded on the assumption that the pictures should follow each other instantaneously, quietly, positively, without jumping, without jerking. Instead of holding a lot of engineering conferences on how to accomplish this without resorting to a costly Geneva movement, we went ahead and actually used the Geneva movement and then made the engineers figure out how they could still keep the selling price down to $98.

And, since the customers were to be asked to lay their dough out for a projector, not just a Geneva movement, we had to insist on 1) sufficient brilliance and evenness of illumination to dispense with room darkening in most cases; 2) safeguarding the filmstrip by automatic separation of the glass pressure plates before it can move; 3) fast setup by virtue of drop-in loading for immediate sprocket engagement, a quick framing lever, and a quick rewind device; 4) cool operation of a 500-w lamp with little or no blower noise and no danger of burning a hole in the film; 5) automatic leveling capable of compensating for unevenness of support.

All this they did, our engineers, muttering all the while that sales people are unreasonable; for they knew all along that not only were they expected to come up with a world-beater of a filmstrip machine but also that the $98 package had to include a mechanism that the user could easily insert when he had Kodachrome slides to show!

To have the audio-visual man show you how well they fulfilled the assignment, drop a line to Eastman Kodak Company, Department 8-AV, Rochester 4, N. Y. As for how you prepare the filmstrips in the first place, you can ask in your note for a little pamphlet of helpful hints we call "Making Filmstrips With Amateur Equipment."

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